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Attorney Docket No. HAR-001DV

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OCT 30 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Strominger et al.
 SERIAL NO.: 08/991,628 GROUP NO.: 1644
 FILING DATE: November 5, 1997 EXAMINER: DiBrino, M.
 TITLE: Preparations for Inducing Immunotolerance and Uses Therefor

Box AF
 Commissioner for Patents
 Washington, D.C. 20231

Sir:

APPLICANTS' BRIEF ON APPEAL TO THE
BOARD OF PATENT APPEALS AND INTERFERENCES

This Appeal Brief is submitted in response to the Notice of Non-Compliance with 37 C.F.R. § 1.192(c) mailed from the United States Patent Office on October 9, 2001. A three-month extension of time for filing this Appeal Brief is respectfully requested.

This Brief in support of an appeal to the Board of Patent Appeals and Interferences from the final rejection of claims 3-6, 11, and 13-16 in the above-referenced application is submitted in triplicate as required by 37 C.F.R. 1.192. Also enclosed are Appendices A-D which are referenced and relied upon herein.

Applicants' Brief on Appeal

U.S. Serial No. 08/991,628

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REAL PARTY IN INTEREST

The Real Party in Interest is the President and Fellows of Harvard College, Cambridge, Massachusetts by virtue of an assignment from the inventors of the parent application, U.S. Serial No. 08/400,796, which issued as U.S. Patent No. 5,874,531. This assignment was recorded at Reel 7540, Frame 0884 on May 15, 1995.

RELATED APPEALS AND INTERFERENCES

The Applicants' undersigned legal representative is unaware of another appeal or interference which will directly affect, or be directly affected by, or have a bearing on the Board's decision in this pending appeal.

STATUS OF CLAIMS

Claims 3-6, 11, and 13-16 are pending in the above-identified application and are the subject of this appeal. Claims 1, 2, 7-10, 12, and 17-29 are cancelled. In response to an election of species requirement, Applicants elected SEQ. ID. NO. 3 as the peptide species and pemphigus vulgaris as the autoimmune disease condition species. Claims 3-6, 11, and 13-16 are set forth in the attached Appendix A.

STATUS OF AMENDMENTS

No amendments were filed subsequent to the Final Office Action mailed from the U.S. Patent Office on July 3, 2000. All previous amendments have been entered.

SUMMARY OF THE INVENTION

Applicants provide the following concise summary of the invention.

The specification states, at page 6, lines 20-31 that: "The present invention provides, in one aspect, pharmaceutical preparations for use in tolerizing individuals to

autoantigens. The preparations include a pharmaceutically acceptable carrier and an isolated human polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. The peptides are not derived from human collagen or human myelin basic protein."

The specification states, at page 7, lines 1-10: "In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals having peptides with this motif are provided. Specific embodiments of the pharmaceuticals include each of the polypeptides described above with respect to pemphigus vulgaris. Thus, methods of tolerizing an individual to a pemphigus vulgaris autoantigen are also provided."

The specification states, at page 7, lines 11-21: "In another set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to antigens of human pathogens which are implicated in human autoimmune disease. The preparations include a pharmaceutically acceptable carrier and an isolated human pathogen polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease."

The specification states, at page 8, lines 1-14: "In another aspect of the invention, pharmaceuticals are provided for vaccination against a human pathogen implicated in the aetiology of autoimmune disease. These pharmaceutical preparations include a pharmaceutically acceptable carrier and an immunogenic preparation effective to immunize against a human pathogen. The human pathogen is one which in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with the autoimmune disease. These

polypeptides are capable of binding to the HLA-DR protein to form a complex which activates T cells which become autoreactive and initiate the autoimmune disease. The preparations of the present invention specifically do not include such polypeptides but, rather, include other antigens from the pathogen."

The specification states, at page 8, lines 14-24: "In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals which lack peptides having this motif are provided. Specific embodiments of the pharmaceuticals include preparations lacking each of the polypeptides described above with respect to pemphigus vulgaris."

Although those teachings are summarized above, the Board is strongly urged to study the specification before considering the rejections on appeal. A copy of the specification is attached as Appendix B. A copy of the parent application, U.S. Serial No. 08/400,796, which issued as U.S. Patent No. 5,874,531 on February 23, 1999, is attached as Appendix C.

ISSUES

1. The first issue presented for appeal is whether claims 3-6 and 11, which directly or indirectly recite a "human polypeptide consisting essentially of an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein" are unpatentable under 35 U.S.C. §112, 1st paragraph as being based on a nonenabling disclosure.

2. The second issue presented for appeal is whether claims 13-16, which directly or indirectly recite a "human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC residues of a sequence motif for an HLA-DR protein" is unpatentable under 35 U.S.C. §112, 1st paragraph as being based on a nonenabling disclosure.

3. The third issue presented for appeal is whether claims 13-16 are unpatentable under 35 U.S.C. §112, 2nd paragraph as being indefinite.

4. The fourth issue presented for appeal is whether the double patenting rejection of claims 3-6 and 13-16 over claim 3 of U.S. Patent No. 5,874,531 is proper.

5. The fifth issue presented for appeal is whether appealed claims 3-5 are novel in view of Amagai et al. (*Autoantibodies against a Novel Epithelial Cadherin in Pemphigus Vulgaris, a Disease of Cell Adhesion*, Cell Vol., 67, 869-877 (1991), hereinafter, "Amagai").

6. The sixth issue presented for appeal is whether appealed claims 13-15 novel in view of Amagai.

7. Although Applicant believes that the above-identified issues correspond to all of the grounds for the pending rejections, Applicants also appeal any other bases for rejection of the pending claims which were not explicitly stated in the Final Office Action, but which may be regarded as still pending.

GROUPING OF CLAIMS

With regard to Issue 1, rejected claims 3-6 and 11 stand or fall together.

With regard to Issue 2, rejected claims 13-16 stand or fall together.

With regard to Issue 3, rejected claims 13-16 stand or fall together.

With regard to Issue 4, rejected claims 3-6 and 13-16 stand or fall together.

With regard to Issue 5, rejected claims 3-5 stand or fall together.

With regard to Issue 6, rejected claims 13-15 stand or fall together.

ARGUMENT

Pursuant to 37 C.F.R. §1.192(c)(8)(iv), the following sections describe antigen presentation, discuss the legal standard applicable to the instant application, indicate the

specific limitations in the rejected claims that are not disclosed in the applied references, and explain how such limitations render the claimed subject matter patentable.

Autoimmune disease is generally defined as the pathological consequences, including tissue injury, produced by autoantibodies or autoreactive T cells interacting with self epitopes. An "epitope" is defined as "an antigenic determinant ... the simplest form or smallest structural area on a complex antigen molecule that can combine with an antibody or T lymphocyte receptor." (The Illustrated Dictionary of Immunology, CRC Press (1995)) Self epitopes are derived from an individual's own proteins. The inappropriate response of the immune system against self epitopes in autoimmune disease can cause serious damage to cells and organs, sometimes with fatal consequences.

The distinction between an antigen and an epitope is critical to the understanding of the present invention. The terms "autoantigen," and "self antigen," as understood in the art and used in the instant specification, refer to a full-length protein or a polypeptide that includes a self epitope which, as stated above, is the simplest form or smallest structural area on a complex antigen molecule that can combine with an antibody or T lymphocyte receptor.

T cell recognition of antigens, including self antigens, involves a tri-molecular complex of (1) a T cell receptor, (2) an MHC molecule, such as HLA-DR, and (3) a short peptide comprising an epitope. (See for example, page 6, lines 27-30, original claims 3 and 13, and pending claims 3 and 13 which require the that the polypeptides of the invention be "capable of binding to HLA-DR protein to form a complex which activates autoreactive T cells in subject having the autoimmune disease.") When the MHC molecule is a Class II molecule, such as HLA-DR, the peptides derived from antigen are heterogeneous in size. (See page 5, lines 8-10.) Peptides that bind HLA-DR molecules have been reported as ranging in size from 12-25 amino acid long. (See Chicz et al., "Predominantly naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size," Nature, 358:764-768 (1992); cited on page 5, at line 10 of the original specification.)

Particular MHC class II molecules (e.g., HLA-DR) have been closely associated with autoimmune disease. For example, HLA-DR4 has been associated with the autoimmune condition pemphigus vulgaris (PV). PV is an autoimmune disease of the skin which is manifested by blistering lesions of the skin and mucous membranes. (See page 2, lines 13-17.)

The present specification teaches methods of identifying peptides that form part of the tri-molecular complex with autoreactive T cells and HLA-DR molecules. Such peptides are useful to treat autoimmune diseases, for example, by inducing high dose tolerance to thereby render autoantigenic T cells unresponsive to self epitope bound to an HLA-DR molecule. (See page 30, lines 14-31 and page 52, lines 11-15.)

A large body of clinical and epidemiological evidence suggests that bacterial or viral infection may trigger the induction of autoimmunity. (See page 3, lines 13-17.) One theory suggests that peptides of a pathogen that closely resembling a self peptides "mimic" self epitopes to activate autoreactive T cells and induce autoimmunity. Accordingly, the present invention also provides for methods of vaccinating an individual against an autoimmune condition by vaccinating the individual against the pathogen with a preparation that specifically excludes autoreactive peptides from the pathogen, that is, peptides that form part of the complex with autoreactive T cells and HLA-DR molecules. (See page 32, lines 9-29.)

Issue 1 – Rejection of Claims 3-6 and 11 Under 35 U.S.C. §112, First Paragraph

Claims 3-6 and 11 were rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to meet the enablement requirement. In particular, the Office Action suggested that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected to make and/or use the invention.

The Office Action states that "the specification, while being enabling for a pharmaceutical preparation comprising a human polypeptide *consisting of* one of SEQ IDS NOS: 1-7, does not reasonably comprise a human polypeptide *consisting essentially*

of an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein, nor *consisting essentially of* one of SEQ ID NOS: 1-7." (Emphasis added.)

Applicants' Rebuttal of the Rejection of Claims 3-6 and 11 under 35 U.S.C. §112, First Paragraph

According to the MPEP 2111.03, the transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. In re Herz, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (Emphasis in original.) Furthermore, "A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." PPG Industries v. Guardian Industries, 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353-54 (Fed. Cir. 1998).

"For search and examination purposes, *absent a clear indication in the specification of what the basic and novel characteristics actually are*, 'consisting essentially of' will be construed as equivalent to 'comprising.'" See, e.g., PPG, 156 F.3d at 1355, 48 USPQ at 1355 (Emphasis added.) However, when there is a clear indication in the specification of what the basic and novel characteristic are, "the "consisting essentially of" terminology would ... *exclude additional unspecified ingredients which would affect the basic and novel characteristics* of the product defined in the balance of the claim." In re Garnero, 162 USPQ 221, 223 (C.C.P.A. 1969) (Emphasis added.)

As explained below, Applicants have clearly identified the "basic and novel characteristics" of the present invention and therefore the "consisting essentially of" phrase should be construed as open to those materials that do not materially affect the basic and novel characteristics and closed to those materials that do materially affect the basic and novel characteristics of the claimed invention.

In brief, the present application teaches methods of "defining those amino acids of the self or non-self antigen that are needed for MHC binding and TCR [T cell receptor] contact" so that "self epitopes involved in autoimmune disease may be identified." (See page 5, lines 24-31.) It is these self epitopes (and non-self epitopes that mimic self epitopes) that are the active agents of the pharmaceutical preparations for tolerization recited in claims 3-6 and 11. Accordingly, the basic and novel characteristics of the claimed polypeptides include the ability (1) bind to HLA-DR protein and (2) activate autoreactive T cells from a subject having an autoimmune disease.

Applicants submit that independent claim 3 (as well as claims 4-6 and 11 that depend from claim 3) which claims polypeptides that "consist essentially of an amino acid sequence corresponding to the core MHC binding residues of the sequence motif for an HLA-DR molecule ... [which] binds to said HLA-DR protein [and] activates autoreactive T cells from a subject having said autoimmune disease" embraces only polypeptides that bind to HLA-DR and activate autoreactive T cells, and specifically excludes peptides that are incapable of binding to HLA-DR and/or activating autoreactive T cells.

Furthermore, the specification expressly states that "peptides including at least the MHC binding and TCR contact residues are contemplated as equivalents." (See page 28, lines 27-30.) Thus, the present specification clearly indicates that the basic and novel characteristics of the claimed polypeptides are the ability to (1) bind to HLA-DR protein and (2) activate autoreactive T cells from a subject having an autoimmune disease. Furthermore, the claims explicitly require that the preparations of the invention comprise polypeptide that "binds to [said] HAL-DR protein" and "activates autoreactive T cells."

For the foregoing reasons, Applicants submit that the instant specification fully enables pending claims 3-6 and 11 when the term "consisting essentially of" is interpreted properly as partially open and partially closed. Under this construction of the "consisting essentially of" phrase, claims 3-6 and 11 include polypeptides with an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for HLA-

DR protein and other components (e.g., additional amino acids) that do not materially affect the ability of the polypeptides to bind to HLA-DR molecules and activate autoreactive T cells from a subject having an autoimmune disease.

Full-length proteins or long polypeptides do not fall within the scope of claims 3-6 and 11 because, as was notoriously well known in the art at the time the application was filed, MHC molecules do not bind full-length proteins or long polypeptides from an antigen. MHC molecules bind short peptides that are small fragments of an antigen. The distinction between an antigen and an epitope is critical to the understanding of the present invention. The terms "autoantigen," and "self antigen," as understood in the art and used in the instant specification, refers to full-length protein or polypeptide that includes a self epitope. As stated above, an "epitope" is an antigenic determinant ... the simplest form or smallest structural area on a complex antigen molecule that can combine with an antibody or T lymphocyte receptor.

The present application is directed to identification of self epitopes and preparations that utilize such self epitopes. The present specification explains that although the target antigens implicated in the immunopathogenesis of disease have been identified, specific self epitopes have not been identified. See, for example, page 23, lines 15-20, which reads: "An ever increasing number of autoimmune diseases are now being associated with particular alleles of the MHC class II HLA-DR locus. For most of these autoimmune diseases, the self epitope remains unknown. For some, however, the self protein involved in autoimmune response is known or suspected. In one aspect of the present invention, a method is provided for identifying the self epitopes involved in autoimmune diseases associated with HLA-DR alleles." (Emphasis added.) The pharmaceutical preparations of claims 3-6 and the method of claim 11 utilize these self epitopes to tolerate (i.e., render autoreactive T cells unresponsive to the self epitope, see page 52, lines 12-17) an individual to an autoantigen through MHC class II presentation.

The specification reiterates the distinction between an autoantigen and self epitope in the description of the autoimmune disease pemphigus vulgaris at page 37, lines 16-23

"Although the autoantigen for pemphigus vulgaris is known, the precise epitopes within the autoantigen have previously remained unknown. Using the methods of the present invention, it has been possible to identify a small set of peptides that may serve as the autoantigenic determinants. The target antigen of pemphigus vulgaris is an epithelial adhesion molecule of the cadherin family, desmolgein 3 (Amagai et al., 1991)." As stated in the Abstract, "The peptides relating to pemphigus vulgaris are self epitopes," thus, the polypeptides recited in claims 3-6 and 11 are essentially self epitopes that form part of the HLA-DR/T cell receptor/peptide complex that causes autoimmune disease and they are necessarily relatively small peptides.

Furthermore, the working examples of the polypeptides of the invention that are disclosed in the instant specification (SEQ ID NO. 1-7) are peptides that are 15 amino acids long, "partly as a result of the computer database search program used (Genetics Computer Group program "Findpatterns") but also *corresponding to the size of the cleft in MHC class II molecules.*" (See page 37, lines 10-14; emphasis added.) Thus, the instant specification teaches that polypeptides capable of binding to HLA-DR proteins may be approximately 15 amino acids long. However, the instant specification also teaches that shorter or longer peptides "may have utility" and thus, "fall within the spirit and the scope of the claims" (See page 28, lines 16-30.) Thus, pending claim 3 which recites a pharmaceutical preparation for tolerization ... consisting essentially of an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA DR protein, ... [which] binds to said HLA-DR protein [and] activates autoreactive T cells" embraces polypeptides that consist of self epitopes (which may be heterogeneous in size) and excludes full length proteins and large protein fragments that are too large to form the HLA-DR/T cell receptor/peptide complex.

The Office Action suggests that "there is no guidance in the specification as to what alterations result in a functional polypeptide, i.e., one that binds HLA-DR." (Emphasis added.) Applicants submit that the present specification provides detailed analysis of the binding pockets of HLA-DR molecules which properly teaches the skilled artisan how to predict which peptides are capable of binding to HLA-DR molecules. For

example, the claims 5 and 15 provide a motif to identify peptides that are capable binding to HLA-DR molecules and activating T cells associated with the autoimmune disease pemphigus vulgaris.

The Office Action also suggests that undue experimentation would be required to determine what substitutions would be acceptable to retain functional activity.

Applicants note that "The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." In re Angstadt, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Moreover, Applicants submit that neither "extensive experimentation" nor "undue experimentation" is required to practice the claimed invention.

The identification of amino acid sequences that have functional activity including the ability to bind to HLA-DR proteins and to activate autoreactive T cells maybe readily determined by various assays that were well known to the skilled artisan at the time the present application was filed. For example, functional activity may determined using the T cell proliferation assay described in Example 1 of the specification at page 44, lines 11-22.

The MPEP 2164.01(a) states: "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." In re Certain Limited-Charge Cell Culture Microcarriers, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983); Massachusetts Institute of Technology v. A.B. Fortia, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985). The specification teaches, at page 25, lines 3-13, that the peptides of the invention may be screened for activity using experimentation that is typical in the art, for example, by "*in vitro* tests for the ability to induce proliferation of autoreactive T cells or to induce the secretion of lymphokines (cytokines) form these T cells or to induce other effector functions such as cytotoxicity." Accordingly, Applicants submit that undue experimentation is not required to practice the claimed invention.

The Office Action cites the Ngo reference ("The Protein Folding Problem and Tertiary Structure Prediction," Merz & LeGrans, Birkhauser, Boston, pages 491-495,

1994) to support the assertion that the relationship between the sequence of a polypeptide and the tertiary structure is not predictable. As is well known in the art, proteins are cleaved into fragments of approximately 12-25 amino acids and bound in the binding cleft of the HLA-DR molecule in an unfolded or essentially linear conformation. See page 116 of Cellular and Molecular Immunology, attached at Appendix D, which states "T cells recognize only linear determinants of peptides defined predominantly by primary amino acid sequences that assume extended confirmations within the peptide-binding clefts of MHC molecules." Thus, the tertiary structure of the peptide does not materially impact the ability of a self epitope polypeptide to bind to HLA-DR molecules and activate autoreactive T cells. Accordingly, Applicants submit that the difficulty in predicting the folding pattern or tertiary structure of full-length proteins discussed in Ngo is irrelevant to the ability of the peptides of the invention to bind to HLA-DR molecules and/or activate autoreactive T cells.

Therefore, in light of the foregoing reasons, Applicants respectfully request that the rejections of claims 3-6 and 11 under 35 U.S.C. §112, first paragraph, be reconsidered and withdrawn.

Issue 2 – Rejection of Claims 13-16 Under 35 U.S.C. §112, First Paragraph

Claims 13-16 were rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to meet the enablement requirement. In particular, the Office Action suggested that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected to make and/or use the invention.

The Office Action alleges that claim 13 is not enabled for preparations for immunizing an individual against a "human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein."

Applicants' Rebuttal to the Rejection of Claims 13-16 under 35 U.S.C. §112, First Paragraph

The Office Action alleges that claim 13 is not enabled for preparations for immunizing an individual against a "human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein."

"Transitional phrases such as "composed of," "having," or "being" must be interpreted in light of the specification to determine whether open or closed claim language is intended. See, e.g., Regents of the Univ. of Cal. v. Eli Lilly & Co., 119 F.3d 1559, 1573, 43 USPQ2d 1398, 1410 (Fed. Cir. 1997), cert. denied, 118 S. Ct. 1548 (1998) (In the context of a cDNA having a sequence coding for human PI, the term "having" still permitted inclusion of other moieties.). MPEP 2111.03

Applicants note that the objected to phrase recited in claim 13 sets forth the target of the immunization, a pathogen that in its native form includes polypeptides having amino acid sequence corresponding to the core MHC binding residues of a sequence motif, however, the final limitation of the claim requires that the pharmaceutical preparation be free of autoantigens (i.e., peptides with amino acid sequence corresponding to the polypeptide that binds to HLA-DR protein and activates autoreactive T cells from a subject having the autoimmune disease). Thus, the epitopes described in the specification and recited in claims 3-6 and 11 are removed from the preparations of claims 13-16 to vaccinate an individual against a pathogen associated with autoimmune disease without exposing the individual at risk to the autoimmune disease to autoantigens that are native to that pathogen.

The specification teaches that preparation for vaccinating a person at risk of an autoimmune disease of the invention may be used to induce immunity against a pathogen associated with autoimmunity without exposing the individual to the autoantigens associated with the pathogen. Thus, the phrase "having" as used in claim 13 is an open transitional phrase, which includes polypeptides native to the pathogen. However, the

"free of the amino acid sequence" corresponding to the core MHC binding residues limitation specifically excludes epitopes that bind to HLA-DR and activate autoreactive T cells.

Applicants submit, for the reasons stated above, that the instant specification properly teaches the skilled artisan how to identify peptides that bind to HLA-DR molecules associated with autoimmune disease and activate autoreactive T cells in a person having such an autoimmune disease. The instant specification further teaches the skilled artisan that an individual at risk of an autoimmune disease, which is associated with a human pathogen, may be immunized against the pathogen with a preparation that may include polypeptides of the pathogen but certianly excludes (i.e., is free of) epitopes that activate autoreactive T cells in a person inflicted with the autoimmune disease.

Therefore, in light of the foregoing reasons, Applicants respectfully request that the rejections of claims 13-16 under 35 U.S.C. §112, first paragraph, be reconsidered and withdrawn.

Issue 3 – Rejection of Claims 13-16 Under 35 U.S.C. §112, Second Paragraph

Claims 13-16 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Office Action suggests that the recital of the phrase "wherein said preparation is free of a polypeptide corresponding to said sequence" is unclear.

Applicants' Rebuttal of the Rejection of Claims 13-16 under 35 U.S.C. § 112, 2nd Paragraph

Applicants submit that claim 13 clearly claims a pharmaceutical for vaccinating an individual at risk of an autoimmune disease by vaccinating against a human pathogen with a preparations which specifically excludes (is free of) autoantigenic peptides, that is,

pathogenic polypeptides corresponding to a sequence motif for HLA-DR protein associated with an autoimmune disease. The text of claim 13 reads as follows:

A pharmaceutical preparation for vaccinating an individual at risk of an autoimmune disease comprising a pharmaceutically acceptable carrier and

an amount of an immunogenic preparation effective to *immunize against a human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein;*

wherein said sequence motif for said HLA-DR protein is based upon the structure of the HLA -DR binding site

wherein said HLA-DR protein is associated with said autoimmune disease;

wherein said polypeptide binds to said HLA-DR protein;

wherein said polypeptide bound to said HLA-DR protein activates autoreactive T cells from a subject having said autoimmune disease; and

wherein said preparation is *free of a polypeptide corresponding to said sequence.* (Emphasis added.)

The Office Action suggests that the phrase "free of a polypeptide corresponding to said sequence" is indefinite. Applicants submit that this phrase is amenable to a single interpretation. specifically, the preparation is free of autoantigenic peptides, that is, peptides that bind to HLA-DR and activate autoreactive T cells are not present in the pharmaceutical preparation.

The Office Action further suggests that the recitation of "includes a polypeptide" is unclear because it is not clear whether said polypeptide is a portion of a protein from a pathogenic organism. This phrase, taken in the context of the limitation "immunize against a human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for

an HLA-DR protein" clearly indicates that the polypeptide recited in claim 13 is a portion of a protein from the pathogenic organism.

Applicants submit that claim 13 satisfies the requirements of 35 U.S.C. §112, second paragraph, respectfully request that the rejection of claim 13 and claims 14-16 (which depend from claim 13) under 35 U.S.C. §112, second paragraph, be reconsidered and withdrawn.

Issue 4 - Double Patenting

Claims 3-6 and 13-16 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 3 of U.S. Patent No. 5,874,531 ("the '531 patent"). The Office Action states that "although the conflicting claims are not identical, they are patentably indistinct from each other because the composition comprising the peptides of claim 3 of the '531 patent are encompassed by the instant claims. Applicants note that the instant application is a division of the application that issued as the '531 patent and, as such, 35 U.S.C. § 121 prohibits the use of the '531 patent as a reference against the instant application. Thus, Applicants respectfully request that the double patenting rejection over claim 3 of the '531 patent be reconsidered and withdrawn.

Issue 5 - Rejections of Claims 3-5 Under 35 U.S.C. §102(b)

Claims 3-5 were rejected under 35 U.S.C. §102(b) as being anticipated by Amagai. The Office Action states that "in the absence in the specification of a definition of a 'human polypeptide consisting essentially of ...', the claim language is open, and inclusive of the full-length autoantigen."

Applicants' Rebuttal to the Rejection of Claims 3-5 under 35 U.S.C. §102(b)

As stated above, with regard the §112, first paragraph rejection, Applicants submit that the specification clearly teaches the basic and novel characteristics of the invention, and the transitional phrase is therefore partially open and partially closed.

The disclosed invention provides a binding motif to determine which residues of a putative antigenic protein are capable of binding autoimmune associated HLA-DR proteins and activating autoreactive T cells. As stated above, independent claims 3 and 13 are intended to embrace polypeptides capable of binding autoimmune associated HLA proteins, not a full-length autoantigenic protein as described in Amagai. Thus, Applicants submit that Amagai fails to teach or even suggest the invention recited in claims 3-5.

Although Amagai teaches that desmoglein 3 is the full length autoantigen for pemphigus vulgaris, Amagai fails to teach or even suggest what short peptides make up the self epitopes for pemphigus vulgaris. Applicants submit that inclusion of the full-length 103 kD pemphigus vulgaris antigen protein disclosed by Amagai in the preparations of claim 3 would materially affect the basic and novel characteristics of the preparation and, therefore, the full-length pemphigus vulgaris protein is excluded from the scope of independent claim 3. Applicants further submit that one of ordinary skill in the art would readily recognize that a preparation for tolerization consisting essentially of an isolated peptide capable of binding to HLA-DR and activating autoreactive T cells is superior to a preparation containing a full-length protein.

In conclusion, Applicants submit that independent claim 3 is not anticipated by Amagai. Applicants further submit that Amagai does not anticipate claims 4 and 5, which depend from claim 3. In light of the foregoing, Applicants respectfully request that the 35 U.S.C § 102(b) rejection of claims 3-5 be reconsidered and withdrawn.

Issue 6 - Rejections of Claims 13-15 Under 35 U.S.C. §102(b)

Claims 13-15 were rejected under 35 U.S.C. §102(b) as being anticipated by Amagai. The Office Action states that "in the absence in the specification of a definition of a 'human polypeptide consisting essentially of ...', the claim language is open, and inclusive of the full-length autoantigen."

Applicants' Rebuttal of the Rejection of Claims 13-15 Under 35 U.S.C. §102(b)

Applicants submit that Amagai fails to anticipate pending claims 13-15 for the following reasons. Independent claim 13 recites, in part, a vaccination preparation "that in its native preparation includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein ... wherein said preparation is free of a polypeptide corresponding to said sequence" amino acid sequence corresponding to a sequence motif for an HLA-DR protein." As stated above, Amagai fails to teach what portions or segments of the desmoglein 3 protein contain the autoantigen for pemphigus vulgaris. Furthermore, Amagai fails to teach a vaccination preparation which includes antigenic polypeptides of a pathogen and excludes polypeptides that activated autoreactive T cells from a subject having an autoimmune disease. Thus, Amagai does not anticipate claims 13-15.

In conclusion, Applicants submit that independent claim 13 is not anticipated by Amagai. Furthermore, Amagai fails to anticipate claims 14-15, which depend from claim 13.

7. The Claimed Invention Is Not Unpatentable Under Any Other Possible Bases

Applicants believe that the foregoing arguments address each of the pending rejections of the pending claims. In particular, the present Brief addresses each of the

rejections made in the Final Office Action. However, if the Examiner regards any of other rejections as currently pending, Applicant requests that any and all such rejections be raised in the Examiner's Answer so that Applicant has an opportunity to respond.

CONCLUSION

For the reasons given above, it is respectfully urged that the final rejections be reversed and the application be passed to issue with claims 3-6, 11, and 13-16.

A Petition and Fee for a three-month Extension of Time for Response, is submitted herewith. Applicants believe that no other fees are necessitated by the present filing. However, in the event that any additional fees are due, the Commissioner is hereby authorized to charge any such fees to Attorney's Deposit Account No. 20-0531.

Respectfully submitted,

Date: February 11, 2002
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Appendix A

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3. A pharmaceutical preparation for tolerization comprising a pharmaceutically acceptable carrier and

an amount of an isolated human polypeptide effective for tolerizing an individual to an autoantigen, said human polypeptide consisting essentially of an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein;

wherein said sequence motif for said HLA-DR protein is based upon the structure of the HLA-DR binding site;

wherein said HLA-DR protein is associated with a human autoimmune disease;

wherein said polypeptide binds to said HLA-DR protein;

wherein said polypeptide bound to said HLA-DR protein activates autoreactive T cells from a subject having said autoimmune disease; and

wherein said polypeptide is a non-collagen and non-myelin basic protein polypeptide.

4. The pharmaceutical preparation of claim 3 wherein said HLA-DR protein is an HLA-DR4 protein and said autoimmune disease is pemphigus vulgaris.

5. The pharmaceutical preparation of claim 4 wherein said motif is PV motif #1.

6. The pharmaceutical preparation of claim 4 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.

11. A method of tolerizing an individual to an autoantigen of pemphigus vulgaris comprising

administering an effective amount of the pharmaceutical preparation of any one of claims 4-6 to a subject in need of such treatment.

Applicants' Brief on Appeal

U.S. Serial No. 08/991,628

Appendix A

13. A pharmaceutical preparation for vaccinating an individual at risk of an autoimmune disease comprising a pharmaceutically acceptable carrier and

an amount of an immunogenic preparation effective to immunize against a human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to the core MHC binding residues of a sequence motif for an HLA-DR protein;

wherein said sequence motif for said HLA-DR protein is based upon the structure of the HLA -DR binding site;

wherein said HLA-DR protein is associated with said autoimmune disease;

wherein said polypeptide binds to said HLA-DR protein;

wherein said polypeptide bound to said HLA-DR protein activates autoreactive T cells from a subject having said autoimmune disease; and

wherein said preparation is free of a polypeptide corresponding to said sequence.

14. The pharmaceutical preparation of claim 13 wherein said HLA-DR protein is an HLA-DR4 protein and said autoimmune disease is pemphigus vulgaris.

15. The pharmaceutical preparation of claim 14 wherein said motif is PV motif #1.

16. The pharmaceutical preparation of claim 14 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.

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IDENTIFICATION OF SELF AND NON-SELF ANTIGENS IMPLICATED IN AUTOIMMUNE DISEASE

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Field of the Invention

The present invention relates to the field of immunology and, in particular, to the identification of self and non-self antigens implicated in human autoimmune responses. The invention relates to methods of identifying such self and non-self antigens and provides examples of such antigens relating to multiple sclerosis and pemphigus vulgaris. The invention also relates to the use of such antigens for *in vitro* assays, animal models, therapeutic agents and vaccines.

Background of the Invention

Human autoimmune diseases have a striking genetic association with particular alleles of major histocompatibility complex ("MHC") class I or class II genes. The field was established by the seminal discovery of HLA-B27 linked susceptibility to ankylosing spondylitis, a chronic inflammatory joint disease (Brewerton et al., 1973; Schlosstein et al., 1973). MHC associated susceptibility has now been documented for a variety of human autoimmune diseases, including insulin dependent diabetes mellitus (IDDM), rheumatoid arthritis (RA), pemphigus vulgaris (PV), multiple sclerosis (MS) and myasthenia gravis (MG), just to name a few (Todd et al., 1987; Ahmed et al., 1990; Ahmed et al. 1991; Lanchbury & Panayi, 1991; Spielman & Nathenson, 1982; Protti et al., 1993).

The MHC locus most commonly associated with autoimmune disease is the HLA-DR β locus (also known as DRB1), a highly polymorphic locus with over fifty known alleles. For example, a large body of epidemiological work has documented the association of rheumatoid arthritis with the DR4 (DRB1*0401,

DRB1*0404) and DR1 (DRB1*0101) alleles, with the DR4 alleles conferring a higher risk than DR1 (Lanchbury & Panayi, 1991). The risk is dramatically increased when the subject is homozygous or heterozygous for DRB1*0401 and/or DRB1*0404. The observation that arthritis is associated with three DR alleles that are structurally similar led to the development of the 'shared epitope' hypothesis as DRB1*0401, 0404 and 0101 share critical polymorphic residues in the DR β 67-71 cluster (Gregersen et al. 1987; Lanchbury & Panayi, 1991). These residues (in particular DR β 71) appear to be critical in defining the selectivity of peptide binding to the disease associated molecules.

Pemphigus vulgaris is an autoimmune disease of the skin in which high titer autoantibody production to an epidermal cell adhesion molecule (desmoglein 3) results in a loss of keratinocyte adhesion (acantholysis) and severe blister formation (Amagai et al., 1991). In different ethnic groups the disease is associated either with a DR4 allele (DRB1*0402) or with a rare DQ1 allele (DQB1*05032); only a small fraction of PV patients have neither susceptibility gene (Ahmed et al., 1991; Ahmed et al., 1990; Scharf et al., 1988b). The DR4 subtype associated with pemphigus differs only at three positions in the DR β 67-71 cluster from the DR4 subtype associated with RA. The PV associated molecule has a negative charge (Glu) at the critical position (DR β 71); the neighboring position (DR β 70) is also negatively charged. The DR4 subtype associated with PV is the only one that carries a negative charge at DR β 71; a positive charge (Arg) is found at DR β 71 in the RA associated DR4 molecules.

With respect to multiple sclerosis, recent immunological studies suggest that myelin basic protein (MBP) may be one of the important target antigens in the immunopathogenesis of the

disease. Several studies have demonstrated that MBP specific T cells are clonally expanded in MS patients and in an in vivo activated state (Allegretta et al., 1990; Wucherpfennig et al., 1994b; Zhang et al., 1994). Reactivity with the immunodominant MBP(84-102) peptide is found predominantly in subjects carrying HLA-DR2 (the most common subtype of which is DRB1*1501), a genetic marker for susceptibility to MS. The MBP(84-102) epitope can also be presented by other MHC class II antigens, including HLA-DQ1 (Ota et al., 1990; Martin et al., 1990; Pette et al., 1990; Wucherpfennig et al., 1994a). In vivo, the T cell response to this peptide appears to be dominated by a few expanded clones.

While associations between MHC alleles and disease states have implicated autoimmunity in the aetiology of these diseases, a large body of clinical and epidemiological evidence suggests that infections may be important in the induction of autoimmunity. For example, particular viral infections frequently precede autoimmune myocarditis and type I diabetes (IDDM) (Rose et al., 1986; Ray et al., 1980). Environmental agents also influence the risk of developing multiple sclerosis as demonstrated by migration studies. Individuals that migrate after age 15 carry the risk for developing MS associated with their geographic origin while individuals who migrate earlier in life acquire the risk of the geographical region to which they migrated (Kurtzke, 1985). These studies are consistent with the hypothesis that a group of pathogens that are relatively ubiquitous in a certain geographic region influence the risk of developing multiple sclerosis (MS). The mechanism(s) leading to clonal expansion of MBP reactive T cells remain to be identified but could involve recognition of viral peptides with sufficient

structural similarity to the immunodominant MBP peptide. The initiation of autoimmunity by such a mechanism could then lead to sensitization to other CNS self antigens by determinant spreading (Lehmann et al., 1992; Kaufman et al. 1993; Tisch et al., 1993). Consonant with this hypothesis, it has been noted that inflammatory CNS disease can follow infection with a number of common viral pathogens, such as measles and rubella. On the other hand, the absence of virus in the CNS of these patients and reactivity to myelin basic protein in these patients suggest an autoimmune mechanism (Johnson et al., 1984).

Efforts to identify sequence homologies between self peptide epitopes that might be involved in autoimmunity and various bacterial and viral pathogens have therefore been made. These homology searches have focused on alignments with sequence identity. No success has been reported using such alignments in identifying epitopes from pathogens that could cross react with presumably pathogenic T cell lines from human patients with autoimmune disease (Oldstone, 1990). A sequence identity was recently found between an epitope in a Coxsackie virus protein and GAD65, suspected of being an autoantigen in diabetes. These peptides could reciprocally generate polyclonal T cell lines from mice that cross react with the other peptides (Tian, et al., 1994). No evidence, however, was provided that these peptides could stimulate clones from diabetic mice (or humans).

Recent developments in the field, in particular the identification of allele specific peptide binding motifs have transformed the field (Madden et al., 1991; Rötschke & Falk, 1991). Based on this knowledge the structural basis for MHC linked susceptibility to autoimmune diseases can be reassessed

at a level of detail sufficient for solving longstanding questions in the field. Motifs for peptide binding to several MHC class I and class II molecules have been defined by sequence analysis of naturally processed peptides and by mutational analysis of known epitopes. MHC class I bound peptides were found to be short (generally 8-10 amino acids long) and to possess two dominant MHC anchor residues; MHC class II bound peptides were found to be longer and more heterogeneous in size (Madden et al., 1991; Rötschke & Falk, 1991; Jardetzky et al. 1991, Chicz et al. 1992, 1993). - Due to the size heterogeneity, however, it has proven more difficult to define MHC class II binding motifs based on sequence alignments. More recently, a crystal structure for HLA-DR \downarrow demonstrated that there is a dominant hydrophobic anchor residue close to the N-terminus of the peptide and that secondary anchor residues are found at several other peptide positions (Brown et al., 1993). Even this work, however, could not provide a detailed description of the binding pockets of HLA-DR proteins, the particular residues involved in the formation of these pockets or the structural requirements or antigens for MHC binding.

In the present disclosure, a detailed description of the HLA-DR antigen binding pockets is provided (Stern et al., 1994). With this information, together with functional information defining those amino acids of the self or non-self antigen that are needed for MHC binding and TCR contact (e.g., Wucherpfennig et al. 1994, 1995), binding motifs for the various HLA-DR allotypes may be developed, self epitopes involved in autoimmune disease may be identified and a method is provided for identifying bacterial and viral epitopes which may initiate a human autoimmune response.

Summary of the Invention

The present invention provides, in one aspect, seven different isolated polypeptides derived from the human desmoglein 3 protein and implicated as self epitopes in the autoimmune disease pemphigus vulgaris (PV). These peptides consist essentially of the seven amino acid sequences disclosed herein and designated SEQ ID NO.: 1 through and SEQ ID NO.: 7. In particular, the invention provides isolated peptides which consist of these sequences, the core MHC binding residues of these sequences, or the inner core MHC binding residues of these sequences.

The present invention provides, in another aspect, eight different isolated polypeptides derived from human pathogens but implicated in the aetiology of the autoimmune disease multiple sclerosis. These peptides consist essentially of the eight amino acid sequences disclosed herein and designated SEQ ID NO.: 8 through SEQ ID NO.: 15. In particular, the invention provides isolated peptides which consist of these sequences, the core MHC binding residues of these sequences, or the inner core MHC binding residues of these sequences.

In another set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to autoantigens. The preparations include a pharmaceutically acceptable carrier and an isolated human polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. The peptides are not derived from human collagen or human myelin basic protein.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals having peptides with this motif are provided. Specific embodiments of the pharmaceuticals include each of the polypeptides described above with respect to pemphigus vulgaris. Thus, methods of tolerizing an individual to a pemphigus vulgaris autoantigen are also provided.

In another set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to antigens of human pathogens which are implicated in human autoimmune disease. The preparations include a pharmaceutically acceptable carrier and an isolated human pathogen polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. In addition, three particular sequence motifs are provided for multiple sclerosis and pharmaceuticals having peptides with at least one of these motifs are provided. Specific embodiments of the pharmaceuticals include each of the polypeptides described above with respect to multiple sclerosis. Thus, methods of tolerizing an individual to a multiple sclerosis foreign antigen are also provided.

In another aspect of the invention, pharmaceuticals are provided for vaccination against a human pathogen implicated in the aetiology of autoimmune disease. These pharmaceutical preparations include a pharmaceutically acceptable carrier and an immunogenic preparation effective to immunize against a human pathogen. The human pathogen is one which in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with the autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates T cells which become autoreactive and initiate the autoimmune disease. The preparations of the present invention specifically do not include such polypeptides but, rather, include other antigens from the pathogen.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals which lack peptides having this motif are provided. Specific embodiments of the pharmaceuticals include preparations lacking each of the polypeptides described above with respect to pemphigus vulgaris. Thus, methods of immunizing an individual against pathogens which may cause pemphigus vulgaris are also provided.

Similarly, pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. Three particular sequence motifs are provided for multiple sclerosis and pharmaceuticals which lack peptides having any of these motifs are provided. Specific embodiments of the pharmaceuticals include preparations lacking each of the polypeptides described above

with respect to multiple sclerosis. Thus, methods of immunizing an individual against pathogens which may cause multiple sclerosis are also provided.

The pharmaceutical preparations for immunizing against pathogens that may cause multiple sclerosis may particularly include inactivated forms of the pathogen in which the polypeptide corresponding to the motif are removed or recombinantly altered. Thus, the invention provides such vaccines in which the pathogen and polypeptide are, respectively, Herpes simplex virus and UL15 protein, Herpes simplex virus and SEQ ID NO.: 8, Adenovirus and Adenovirus ORF protein, Adenovirus and SEQ ID NO.: 09, Pseudomonas aeruginosa and phosphomannomutase protein, Pseudomonas aeruginosa and SEQ ID NO.: 10, Papillomavirus and L2 protein, Papillomavirus and SEQ ID NO.: 11, Epstein-Barr virus and DNA polymerase protein, Epstein-Barr virus and SEQ ID NO.: 12, Influenza virus and hemagglutinin protein, Influenza virus and SEQ ID NO.: 13, Reovirus and sigma 2 protein, Reovirus sand SEQ ID NO.: 14, Herpes simplex virus and DNA polymerase, and Herpes simplex and SEQ ID NO.: 15.

The present invention also provides general methods for evaluating a peptide for an ability to induce an autoimmune response. These methods involve choosing an MHC HLA-DR molecule associated with the autoimmune response, selecting at least two major MHC binding pockets of the HLA-DR molecule, identifying sets of amino acid residues which bind within each of the selected pockets, developing a sequence motif for the HLA-DR molecule in which the sets of amino acids define the allowed amino acids at the corresponding positions of the motif, and then comparing the amino acid sequence of the peptide to the sequence motif. Peptides which match the motif

have a much greater likelihood of inducing the autoimmune disease. In addition, if there is a known epitope implicated in the disease, the method may further include selected at least one TCR contact residue of the epitope, identifying a set of amino acid residues which may serve as the TCR contact, and including this set in the motif at the appropriate position. In preferred embodiments, the motifs include restrictions on the residues at positions corresponding to at least the P1 MHC binding pocket and at least one of the P4 and P6 pockets.

In another embodiment of the invention, methods are provided specifically for identifying foreign antigens implicated in human autoimmune response. These methods include the same steps as the previously described methods, but further include a comparison of the resulting sequence motif to sets of human pathogens. In preferred embodiments, peptide sequences from one or more species in the normal human intestinal flora are excluded from consideration. In another preferred embodiment, sequences from one or more species of pathogen which is negatively correlated with the incidence of the disease are excluded. In a most preferred embodiment, the human pathogen peptides are searched and evaluated on a computer database using the motif as a search criterion.

Brief Description of the Drawings

Figure 1 illustrates the pockets in the HLA-DR1 peptide binding. The upper right shows a top view of the molecular surface (1.5 Angstrom probe radius) of the HLA-DR1 peptide-binding site with the C α trace of the HLA-DR1 shown as thin lines. Pockets that accomodate the peptide side chains of an antigen (in this case Influenza virus

hemagglutinin) are shown in detail in surrounding views, and are numbered as described herein. The P1 pocket accommodates the Tyr (308) of the antigen. The P4, P6, P7 and P9 pockets bind antigen residues Gln (311), Thr (313), Leu (314) and Leu (316) respectively. Antigen peptide side chains and the nearby peptide main chain are represented by CPK models and HLA-DR side chains in contact with the peptide are indicated as stick models. Pockets are viewed in the plane of the peptide binding site, towards the N terminus of the peptide (P1 and P6), towards the C terminus of the peptide (P4); towards the β_1 helical region (P7) or towards the α_1 helical region (P9). A full color version of this figure may be found in Stern et al., 1994.

Detailed Description of the Invention

The present invention relates to a method of identifying and evaluating peptides for their ability to induce an autoimmune response or to cause autoimmune disease. In particular, the invention relates to methods of (1) evaluating self peptides for their potential involvement in autoimmune disease when the self epitope or autoantigen is unknown and (2) evaluating foreign peptides for their possible involvement in the aetiology of autoimmune disease. The invention also relates to specific peptides identified by the methods of the invention and representing self and foreign antigens implicated in, respectively, pemphigus vulgaris and multiple sclerosis.

The methods rely upon the development of amino acid sequence motifs to which potential self or foreign epitopes may be compared. Each motif describes a finite set of amino acid sequences in which the residues at each (relative)

position may be (a) restricted to a single residue, (b) - - allowed to vary amongst a restricted set of residues, or (c) allowed to vary amongst all possible residues. For consistency in this disclosure, but without limiting the invention in any way, these sequence motifs will be symbolized as strings of characters in which (a) a position which is restricted to a single residue will be represented by the one-letter abbreviation for that residue, (b) a position which is allowed to vary amongst a set of residues will be represented by a column of the one-letter abbreviations for those residues, and (c) a position which is allowed to vary amongst all amino acid residues will be represented by an "X." As an example only, a motif might specify that the residue at a first position may be any one of the residues valine, leucine, isoleucine, methionine, or phenylalanine; that the residue at the second position must be histidine; that the residue at the third position may be any amino acid residue; that the residue at the fourth position may be any one of the residues valine, leucine, isoleucine, methionine, phenylalanine, tyrosine or tryptophan; and that the residue at the fifth position must be lysine. Such a motif would be represented by the following character string:

V	H	X	V	K
L			L	
I			I	
M			M	
F			F	
			Y	
			W	

In one aspect of the present invention, sequence motifs are developed by analysis of the binding domains or binding pockets of major histocompatibility complex HLA-DR proteins and/or the T cell receptor ("TCR") contact points of epitopes

bound to MHC molecules. By providing a detailed structural analysis of the HLA-DR residues involved in forming the MHC binding pockets, one is enabled to make predictions of sequence motifs for binding to any of the HLA-DR proteins.

In another aspect of the present invention, sequence motifs developed by the methods disclosed herein may be used to identify self peptide epitopes involved in an autoimmune response when the autoantigen is known or suspected.

In another aspect of the present invention, methods of identifying foreign peptide epitopes implicated in autoimmune disease are provided. These methods involve the use of MHC and/or TCR binding motifs to identify peptides derived from certain classes of organisms or pathogens which may initiate human autoimmune response. In this aspect, the motifs may be developed according to the methods of the present invention or by other means known in the art.

Using these sequence motifs as search, evaluation, or design criteria, one is enabled to identify classes of peptides which have a reasonable likelihood of binding to a particular MHC molecule and of interacting with a T cell receptor to induce T cell and/or autoimmune response. Use of these motifs, as opposed to pure sequence homology (which excludes many peptides which are antigenically similar but quite distinct in sequence) or sequence homology with unlimited "conservative" substitutions (which admits many peptides which differ at critical highly conserved sites), represents a significant advance in the ability of one of ordinary skill in the art to evaluate particular peptides for potential involvement in autoimmune disease and to search computer databases of peptide sequences to identify self and foreign peptides which may be involved in autoimmune

response. In addition, the use of MHC and/or TCR binding motifs to search limited databases for foreign peptides which may be implicated in the aetiology of autoimmune disease is a novel application of the concept of binding motifs.

Detailed examples of the practice of the present invention are presented below. The methods of the invention have now been used to identify the previously unknown self peptide epitopes involved in the autoimmune disease pemphigus vulgaris. In addition, the methods have been used to identify a class of foreign peptides which may be involved in the initiation of autoimmune response in multiple sclerosis (for which the self epitope had previously been identified).

Thus, in another embodiment, the invention provides these peptides, in isolated form, which may be used in various diagnostic and therapeutic methods and products alluded to below.

I. The MHC Class II HLA-DR Molecular Mimicry Motif

The HLA-DR binding site is characterized by five major pockets which may bind the amino acid side chains of antigens (Stern et al., 1994, the entire disclosure of which is incorporated herein by reference). See Figure 1. The amino acid residue of the antigen which binds in the first major pocket is designated P1. The remaining residues may then be numbered by their positions relative to P1 (with positive numbers increasing toward the carboxy terminus and negative numbers increasing toward the amino terminus):

P-i ... P-1 P1 P2 P3 P4 ... Pj

Thus, the first major pocket of an HLA-DR molecule, by definition, binds the side chain of residue P1 on an antigen. The remaining major pockets bind residues P4, P6, P7 and P9. These residues are defined as the major MHC contact residues.

The amino acid side chains of residues P-1, P2, P3, P5, P8, and P11 are oriented away from the HLA-DR binding site and, therefore, are available as contact residues for a T cell receptor (TCR). All of these residues are defined as TCR contact residues.

A. The MHC Contact Residues

The first major pocket of the HLA-DR molecule is strongly hydrophobic. It is formed by a stretch of residues at about positions 85, 86, 89 and 90 of the β chain, a stretch of residues at about positions 31, 32 and 34 of the α chain, and side chains from residues at about positions 7 and 43 of the α chain. For example, in HLA-DR1 (DRA, DRB1*0101), the first pocket is formed by residues β 85 (Val), β 86 (Gly), β 89 (Phe), β 90 (Thr), α 31 (Ile), α 32 (Phe), α 34 (Phe), α 7 (Ile), and α 43 (Trp). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992, incorporated by reference herein) and are available through genetic databases.

Although most of the residues that shape the P1 pocket are from the highly conserved DR α chain, the size and nature of this pocket varies due to polymorphisms in the β chain residues involved in the pocket. For the DRB1*0101 protein, the pocket is large and hydrophobic and can accommodate any of the aliphatic or aromatic residues. Polymorphism at the β residues, however, may alter the binding capacity of the P1 pocket. For example, the β 86 residue is known to be polymorphic. Most commonly, this site is occupied by either

Gly or Val. Generally, when Gly is present at β 86 (as in DRB1*0101), any of the aliphatic or aromatic residues may bind within the pocket. When Val is present, however, the pocket is smaller and Tyr and Trp cannot be accommodated. Thus, when β 86 is Gly, position P1 of the molecular mimicry motif may consist of residues chosen from V, L, I, A, M, F, Y, W and when β 86 is Val, position P1 of the motif may consist of residues chosen from V, L, I, A, M, F. Similar considerations apply to the other β residues of the P1 pocket.

The P4 pocket of HLA-DR molecules is also a relatively large, shallow, hydrophobic pocket oriented across the antigen binding site. This pocket can bind a variety of large aliphatic side chains which can maintain hydrophobic interactions along the side and floor of the pocket. The pocket is formed by a stretch of residues at about positions 70, 71, 74 and 78 of the β chain, and side chains from residues at about position 13 of the β chain and about position 9 of the α chain. For example, in HLA-DR1 (DRA, DRB1*0101), the P4 pocket is formed by residues β 70 (Gln), β 71 (Arg), β 74 (Ala), β 78 (Tyr), β 13 (Phe), and α 9 (Gln). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

Like the P1 pocket, the P4 pocket is largely hydrophobic but its binding capacity is affected by polymorphisms at the β residues involved in the pocket. For example, different DR alleles have differently charged residues at position β 71: In DRB1*0404, β 71 is occupied by a positively charged Arg residue whereas in DRB1*0402 β 71 is a negatively charged Glu residue. Thus, although this pocket can generally bind a variety of aliphatic or aromatic side chains (e.g., V, L, I, A, M, F, Y,

W), positively charged P4 antigen residues are disfavored when β_{71} is positively charged and, similarly, negatively charged P4 residues are disfavored when β_{71} is also negative. Similar considerations apply to the other β residues of the P4 pocket. Note that some residues may be involved in forming each of two adjacent pockets (e.g., β_{13} in the P4 and P6 pockets) and therefore the occupancy of one of these pockets by a particular amino acid may influence the occupancy of the other.

The P6 pocket of HLA-DR molecules is a relatively shallow pocket with a preference for smaller (e.g., A, G) P6 antigen residues. The pocket is formed by the highly conserved α_{11} , α_{62} , α_{65} and α_{66} residues and the highly polymorphic β_{11} and β_{13} residues of the HLA-DR protein. For example, in HLA-DR1 (DRA, DRB1*0101), the P6 pocket is formed by residues α_{11} (Glu), α_{62} (Asn), α_{65} (Val), α_{66} (Asp), β_{11} (Leu) and β_{13} (Phe). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

Although there are only two β chain residues in the P6 pocket, they vary widely amongst the DR alleles. With a large Phe residue at β_{13} (as in DRB1*0101), the P6 residue is - preferably one of the small residues (e.g., A, G). In other DR alleles, however, β_{13} is occupied by smaller or more polar residues such as the β_{13} (His) of DRB1*0401. For such alleles, the P6 motif may include somewhat larger and polar residues (e.g., S, T, V) but should still avoid the largest and aromatic residues. Finally, in some alleles, β_{11} and β_{13} are both serine residues (e.g., DRB1*1101) and for these cases more hydrophilic or hydrogen bonding residues may be included in the motif.

The P7 pocket of HLA-DR molecules is also a relatively shallow pocket. The pocket is formed by five residues of the β chain: β 28, β 47, β 61, β 67 and β 71. For example, in HLA-DR1 (DRA, DRB \star 0101), the P7 pocket is formed by residues β 28 (Glu), β 47 (Tyr), β 61 (Trp), β 67 (Leu) and β 71 (Arg). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases. This pocket does not appear to contribute greatly to the specificity of HLA-DR1 but may be important in other alleles.

The P9 pocket of HLA-DR molecules is generally a small hydrophobic pocket and, therefore, small hydrophobic residues are preferred at the P9 position of the antigen. This pocket is formed by the conserved α chain residues α 69, α 72, α 73 and α 76 and by the polymorphic β chain residues β 9 and β 57. For example, in HLA-DR1 (DRA, DRB1 \star 0101) the P9 pocket is formed by α 69 (Asn), α 72 (Ile), α 73 (Met), α 76 (Arg), β 9 (Trp) and β 57 (Asp). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

The P6, P7 and P9 pockets appear to be less important than the P1 and P4 pockets in binding to DR molecules but they may be more important in binding to other isotypes (e.g., the P9 pocket of DQ may be important).

B. The TCR Contact Residues

When there is no known or suspected antigen involved in an autoimmune response, the positions of the sequence motif corresponding to the TCR contact residues may be left unrestricted. That is, absent a known or suspected antigen, the TCR contact positions of the motif are preferably allowed to vary amongst all of the amino acids.

When, on the other hand, there is a known or suspected antigen involved in an autoimmune response, at least some of the motif positions corresponding to the TCR contact residues may be restricted according to the sequence of the antigen. Thus, for example, the P2 and/or P3 and/or P5 positions of the motif may be restricted to only those residues found at the corresponding positions of the antigen. Alternatively, at least some of the TCR contact residues of the motif may be restricted not just to the corresponding residues of the antigen but may be allowed to vary amongst similarly charged and/or structurally similar residues (e.g., K and R). It should be noted, however, that greater conservatism with respect to the TCR contact residues of the motif is justified by the presumably greater specificity of TCR binding relative to the known promiscuity of MHC binding.

C. Developing an HLA-DR Sequence Motif

Given the present disclosure of the HLA-DR residues involved in the formation of the P1, P4, P6, P7 and P9 MHC binding pockets, and given the nucleotide or corresponding amino acid sequence of any particular HLA-DR allele, one is now enabled to develop a sequence motif useful in evaluating or predicting the ability of peptides to bind to that MHC protein. When a particular antigen is known to (or is suspected of) binding to the MHC protein, the TCR contact residues of that antigen may also be considered in the motif.

The method first requires the selection of two or more of the MHC binding pockets for which the choice of peptide residues will be restricted at the corresponding positions of the motif. One may select all five of the major binding pockets and develop a motif in which the corresponding five positions of the motif are restricted or one may select fewer

and develop a less restricted motif. As will be obvious to one of ordinary skill in the art, a more restricted motif will identify a lesser number of peptides in a database search and a less restricted motif will identify a greater number of peptides. In all instances, at least two of the major binding pockets should be selected. When fewer than all five MHC binding pockets are selected, it is preferred that at least one is P1 and that a second is chosen from P4, P6 and P9.

Either before or after the pockets to be restricted by the motif are selected, the set of amino acid side chains likely to bind within each of those pockets and, therefore, the set of amino acid residues that will define the corresponding positions of the motif, must be determined. This may be accomplished by one of ordinary skill in the art by considering the amino acid residues which form the pocket. These residues, identified in Section A above, will determine the size and nature (i.e., hydrophobic, hydrophilic, positively charged, negatively charged, uncharged) of the pocket and consequently, the side chains which may bind within the pocket. Reference may be had to Figure 1 during these considerations but will become increasingly unnecessary as one develops familiarity with the variations of the pockets.

As a general matter, in light of the identification of the residues forming the MHC binding pockets of the HLA-DR proteins disclosed herein, one of ordinary skill in the art can easily develop a sequence binding motif for any HLA-DR protein for which these residues are known for two or more binding pockets. The major considerations are size, hydrophobicity and charge. In light of the present disclosure, each of these considerations may be addressed according to well-known principles. A baseline is disclosed

herein for each pocket for the DRB1*0101 allele. Relative to this HLA-DR protein, one of ordinary skill is enabled to develop motifs for other HLA-DR alleles. Thus, substitutions which lead to larger/smaller pockets suggest that the corresponding motif positions should be restricted so as to permit smaller/larger residues. Similarly, more/less hydrophobic pockets suggest that the corresponding motif positions should be restricted to more/less hydrophobic residues. Finally, positively/negatively charged pockets suggest that positively/negatively charged residues should be excluded and negatively/positively charged residues may be included at the corresponding motif positions. As noted above, the present disclosure enables one of ordinary skill to develop motifs based upon well-established principles.

For example, and not by means of limitation, consider the P1 pocket of the HLA-DR protein. The residues forming this pocket in the DRB1*0101 were described above. For DRB1*0101, the P1 pocket is large and hydrophobic and can accommodate any of the aliphatic or aromatic residues (e.g., V, L, I, A, M, F, Y, W). For the DRB1*1602 protein the same is true. On the other hand, in the DRB1*1501 protein, the B86 position is occupied by Val instead of the Gly found in DRB1*0101 and DRB1*1602. This substitution decreases the size of the P1 pocket in this MHC protein and, as a result, the pocket cannot easily accommodate Tyr or Trp side chains. Thus, for DRB1*1501, the sequence motif at position P1 may be restricted to residues chosen from V, L, I, A, M and F.

Similarly, in light of the present disclosure, one of ordinary skill in the art may consider each of the MHC binding pockets, or only selected pockets, and develop a sequence motif for any HLA-DR protein for which the residues involved

in pocket formation are known. These residues will determine both the size and nature of the pocket and, thereby, the size and nature of the residues which may bind within it. When the pocket is relatively small, the largest amino acid residues (e.g., Y, W) may be excluded from the corresponding position of the motif and, when the pocket is charged, amino acid residues of the same charge may be excluded.

If a self or foreign epitope involved in immune response is known or suspected, and particularly if its TCR contact residues can be defined through the use of responsive T cell clones, the TCR contact residues of the epitope may also be considered in developing a sequence motif. As with the MHC contact residues, all or merely some of the TCR contact residues may be restricted in the motif. And, as with the MHC positions, the restriction of more positions (or the greater restriction of any one position) will result in the identification of fewer peptides in a database search. Unlike the MHC contact residues, for which at least two positions should be restricted in the motif, it is acceptable to omit any restrictions of TCR contact residues in the motif.

If any TCR contact residue positions are restricted in the sequence motif, it is preferred that a position selected from positions P2, P3 and P5 be chosen. Because, in contrast to the relative promiscuity of MHC binding pockets, TCR contact residues appear to have greater specificity, it is preferred that any TCR contact residue positions which are restricted in the motif be rather narrowly restricted. That is, it is preferred that such positions be restricted to just the residue found at the corresponding position of the known antigen or just to residues which are highly similar in structure and charge.

For example, and as further detailed below, the MBP (85-99) peptide is known to be the autoantigen implicated in multiple sclerosis. The P3 residue of this peptide is Phe (residue 91 of MBP) and the P5 residue is Lys (residue 93 of MBP). Thus, if P3 is restricted in the motif, it is preferred that it be restricted to F or, possibly, to F and Y. Similarly, if P5 is restricted, it is preferred that it be restricted to only K or to K and the similarly charged R. Alternatively, P3 and P5 may be left unrestricted.

Obviously, MHC and TCR positions not selected for restriction may be represented by, in the notation of this disclosure, an X. Similarly, as shown in the examples below, several motifs may be developed with varying numbers of positions restricted to varying extents.

II. Identifying Self Epitopes Using Sequence Motifs

An ever increasing number of autoimmune diseases are now being associated with particular alleles of the MHC Class II HLA-DR locus. For most of these autoimmune diseases, the self epitope remains unknown. For some, however, a self protein involved in autoimmune response is known or suspected.

In one aspect of the present invention, a method is provided for identifying the self epitopes involved in autoimmune diseases associated with HLA-DR alleles. That is, by comparing human peptide sequences to the sequence motifs of the present invention, one is enabled to identify those peptides which have the highest likelihood of being the self epitopes involved in the disease.

The method may be applied to any of the autoimmune diseases for which an association with a particular HLA-DR allele is known and for which the amino acid residues forming

the MHC binding pockets (or at least two of the major pockets) for that allele are known. In accordance with the method discussed herein, one may then develop one or more sequence motifs for the HLA-DR protein associated with the disease. Naturally, if the disease is associated with two or more alleles, motifs may be developed for the two or more HLA-DR proteins and, in particular, a consensus motif may be developed using the residues shared by each position of these motifs.

The sequence motif or motifs thus developed are then compared to appropriate sets of human peptide sequences. The human peptide sequences may include all known human sequences or may be limited in ways which will be obvious to one of ordinary skill in the art. For example, if the disease is restricted to particular tissues, the search may be limited to peptides found in those tissues. Conversely, peptides which are also found in unaffected tissues may be eliminated from the search pool. In the most extreme case, when the autoantigen is known or suspected but the particular epitope is unknown, the search may be limited to sequences within the autoantigen (see Example 1).

This method may be used to identify a set of peptides which match the motif and which are most likely to be self epitopes. By varying the number of positions restricted by the motifs, and/or the extent of restriction at each position, and/or the size of the search pool, the number of peptides in the set will, in all likelihood, also be varied. As noted above, at least two of the MHC contact positions (e.g., P1 and P4) should be restricted. Depending upon the number of peptides in the resultant set, a more or less restrictive motif may then be employed to reduce or expand the set. The

desired size of the resultant set depends, of course, upon the subsequent intentions of the practitioner of this method.

Once a set of peptides has been identified, these peptides may optionally be screened for activity. The choice of such screens is at the discretion of the practitioner and beyond the scope of the present invention. Preferred screens, however, include *in vitro* tests for the ability to induce the proliferation of autoreactive T cells or to induce the secretion of lymphokines (cytokines) from these T cells or to induce other effector functions such as cytotoxicity. In some circumstances, human *in vivo* tests may be appropriate and in other circumstances animal models of the human disease may be available.

III. Identifying Foreign Epitopes Implicated in Human Autoimmune Disease

As noted in the Background section, epidemiological evidence has suggested that various bacterial and viral pathogens may be implicated in human autoimmune diseases and the concept of molecular mimicry pervades the literature (reviewed by Oldstone, 1990). Prior attempts to identify particular foreign epitopes involved in human autoimmune disease, however, have depended upon direct sequence similarity to known human epitopes. The results have been disappointing and, to date, no pathogens or peptides derived from pathogens have been shown to be a primary cause of human autoimmune disease.

Thus, in another aspect of the present invention, a method of identifying foreign epitopes implicated in human autoimmune disease is provided. That is, for the first time a method of identifying such foreign epitopes is provided which employs

sequence motifs to identify foreign peptides which have the highest likelihood of being involved in the aetiology of human autoimmune disease.

The method may be applied to any of the autoimmune diseases for which an association with a particular MHC protein is known and for which either (1) a sequence motif has been developed by prior art methods or (2) a sequence motif may be developed by the methods of the present invention. When the self epitope is known or suspected, TCR contact residues may be included in the motif. As before, one or more motifs may be employed and differently derived motifs may be combined to develop consensus motifs.

The sequence motif or motifs thus developed may then be compared to appropriate sets of peptide sequences derived from human pathogens. This is most conveniently accomplished using genetic databases widely available to those of skill in the art. In a most preferred embodiment, the search pool is limited in one or more of the following ways: (1) only sequences from human bacterial or viral pathogens are included; (2) sequences from the normal human intestinal flora (e.g., E. coli or other Enterobacteriaceae) are excluded; and (3) sequences from pathogens are included/excluded depending upon whether the geographical or epidemiological incidence of the pathogens are positively/negatively correlated with the incidence of the autoimmune disease in question (see Example 2).

This method may be used to identify a set of foreign peptides which match the motif and which are most likely to be involved in the human disease. As before, the number of peptides in the set can be varied by using more or less restrictive motifs and/or by varying the search pool. And, as

before, the resultant set of peptides may subsequently be subjected to any of a variety of known screens for activity.

IV. Self and Foreign Epitopes Identified by the Methods of the Present Invention

As detailed in the examples below, the methods of the present invention have been employed (1) to identify seven self epitopes of the desmoglein 3 protein implicated in pemphigus vulgaris and (2) to identify eight foreign epitopes from human pathogens implicated in multiple sclerosis.

Each of these peptides is fifteen residues in length, partly as a result of the computer database search program used (Genetics Computer Group program "Findpatterns") but also corresponding to the size of the cleft in MHC class II molecules. The fifth position of each corresponds to the P1 residue of the antigen. Thus, the P-2 to P11 residues which span the MHC Class II binding cleft correspond to the third through fifteenth residues of these sequences. The P-1 to P9 residues which are important to MHC and TCR binding correspond to the fourth through thirteenth positions. The most important residues for MHC and TCR binding, P-1 to P6 correspond to the fourth through tenth positions of these sequences.

SEQ ID NO.: 1 through SEQ ID NO.: 7, also shown in Table 1 as PVA.1 through PVA.7, correspond to residues 78-93, 97-111, 190-204, 206-220, 251-265, 512-526 and 762-786 of the human desmoglein 3 protein. These peptides are implicated as self epitopes in pemphigus vulgaris. Already, as described in Example 1, two of these peptides have been shown to cause proliferation of T cells isolated from two patients with pemphigus.

SEQ ID NO.: 8 through SEQ ID NO.: 15, also shown in Table 2 are internal fragments of the herpes simplex virus UL15 protein, adenovirus Type 12 ORF, Pseudomonas aeruginosa phosphomannomutase, human Papillomavirus Type 7 L2 protein, Epstein-Barr virus DNA polymerase, Influenza Type A hemagglutinin protein, Reovirus Type 3 sigma 2 protein, and herpes simplex DNA polymerase, respectively. These peptides are implicated as foreign epitopes involved in the aetiology or in remissions of multiple sclerosis. As detailed in Example 2 below, each has been shown to be capable of inducing the proliferation of autoreactive T cell clones isolated from human multiple sclerosis patients. The sequence of the MBP (85-99) peptide is disclosed as SEQ ID NO.: 16.

Each of these proteins has a variety of utilities and, therefore, in another aspect, the present invention provides each of these peptides in isolated form. In addition to the sequences of fifteen residues shown in the Sequence Listing and Tables, the present invention also embraces the fragments of these peptides corresponding to the MHC binding domain. In particular, the invention provides peptides corresponding to the P-2 to P11, P-1 to P9 and P-1 to P6 positions of each of SEQ ID NO.: 1 through SEQ ID NO.: 15. As will be obvious to one of ordinary skill in the art, however, any fragment of any of these peptides which includes at least the P1 and P4 or at least the P1 and P6 or at least the P4, P6 and P7 residues may have utility and is intended to fall within the spirit and scope of the claims. In particular, longer peptides including these peptides or peptides including at least the MHC binding and TCR contact residues described above are contemplated as equivalents.

The manner of production of these peptides is inconsequential but they may be isolated and purified from their natural sources or they may be synthesized. Because of their relatively short length, it is presently contemplated that they should be produced by synthesis. Methods of isolation, purification and synthesis of such peptides are well known in the art and need not be recited herein.

The present invention also provides products and methods using other peptides which may be identified by the methods of the present invention. These peptides, as well as those disclosed above, may be used in each of the following embodiments.

The peptides of the present invention may be used for *in vitro* assays to aid in the diagnosis and classification of pemphigus vulgaris and multiple sclerosis. For example, autoreactive T cells from patients with PV and MS may be tested, as in the examples below or by other known assays, for reactivity with these peptides. The ability or inability of these peptides to cause proliferation of the T cells will, in the case of pemphigus, allow for a refinement of the diagnosis by particular desmoglein 3 epitopes and, in the case of multiple sclerosis, allow further classification of the disease by cross-reactive (self and foreign epitope) types. Immune response to these peptides prior to onset of disease may also be used as an indication of susceptibility or predisposition although care should be taken so as not to induce autoantigenic response.

The peptides of the present invention may also be used in the development of animal models by immunizing animals (e.g., mice, rabbits, non-human primates) with these peptides. Animals which not only develop a response to the peptide but

which also develop an autoimmune disease corresponding to the human pathology will have obvious utility as models for the human disease. Animals which develop a response to the peptide without developing any corresponding autoimmune disease, will have utility as subjects for experiments involving the selective depletion of T cells or other forms of desensitization or tolerization.

Importantly, these peptides and amino acid analogs of these peptides will have utility as therapeutic and diagnostic agents. The pathogens, viruses or bacteria from which they are derived will have utility as vaccinating agents. Some examples of the utility of these materials include the following.

The peptide may be administered in high doses to produce high dose tolerance. This process of tolerization is described in, for example, PCT patent application US93/08456 (International publication number WO 94/06828). Thus, in one set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to autoantigens. The preparations may include a pharmaceutically acceptable carrier and an isolated human polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. By using the peptides disclosed herein or identified by the methods of the invention, such pharmaceuticals can be used to combat autoimmune response. The use of such tolerization to human autoimmune diseases is known in the art and need not be elaborated upon here. Tolerizing doses of

collagen for rheumatoid arthritis and myelin basic protein for multiple sclerosis have been used. The present invention therefore specifically does not embrace these proteins. Other peptides, however, may now be identified by the present methods and be similarly used to treat autoimmune disease.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, using PV motif #1, pharmaceuticals having peptides with this motif are provided. In most preferred embodiments, the pharmaceuticals include at least one of the polypeptides of SEQ ID NO. 1 through SEQ ID NO.:7. Thus, methods of tolerizing an individual to a pemphigus vulgaris autoantigen are also provided.

In a similar set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to antigens of human pathogens which are implicated in human autoimmune disease. The preparations include a pharmaceutically acceptable carrier and an isolated human pathogen polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. Thus, by tolerizing an individual to these antigens, T cells which are cross-reactive with the self antigen will be rendered unresponsive or anergized and protection from the disease will be afforded.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple

sclerosis. In addition, using the three MS motifs disclosed herein, pharmaceuticals having peptides with at least one of these motifs are provided. Specific embodiments of the pharmaceuticals include at least one of the polypeptides disclosed as SEQ ID NO.:8 through SEQ ID NO.:15. Thus, methods of tolerizing an individual to a multiple sclerosis foreign antigen are also provided.

In set of embodiments, pharmaceuticals are provided for vaccination against a human pathogen implicated in the aetiology of autoimmune disease. These pharmaceutical preparations include a pharmaceutically acceptable carrier and an immunogenic preparation effective to immunize against a human pathogen. The human pathogen is one which in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with the autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates T cells which become autoreactive and initiate the autoimmune disease. The preparations of the present invention specifically do not include such polypeptides but, rather, include other antigens from the pathogen. That is, a vaccine is produced which specifically does not include polypeptides which correspond to the sequence motifs for the HLA-DR protein and, if known, the TCR contacts of the self epitope. Because pathogens present a wide array of antigenic determinants, one may eliminate those which correspond to the relevant sequence motif and produce a vaccine which is effective against the pathogen but which will not include peptides implicated in the autoimmune response.

Such vaccines, lacking peptides corresponding to the sequence motifs of the present invention, may be made in any convenient manner by one of ordinary skill in the art. For

example, when producing an influenza vaccine, one may compare the peptide sequences of the influenza virus to sequence motifs developed according the present invention. The vaccine may then be made excluding the proteins which have the motif sequences (e.g. by using a fragment of the virus) or recombinant techniques may be used to produce a virus in which the sequences corresponding to the motif are altered such that they do not match the motif. In preferred embodiments, the altered residues are the TCR contact residues and, in particular, a substitution which alters the charge of a TCR contact residue is preferred. Similar vaccines can be developed for bacterial pathogens using, for example, only portions of the bacterium (e.g. bacterial surface proteins or membrane associated proteins) which lack a motif associated with human autoimmune disease or, again, genetically altering the vaccine bacteria so as to change residues.

The motifs which are considered in creating such vaccines may be chosen on any of several grounds. If the pathogen to which the vaccine is directed is associated with an autoimmune disease, a motif may be developed according to the methods described herein for an HLA-DR protein associated with the disease. If there is also a known or suspected autoantigen, the motif may include TCR contact residues of the self epitope. The pathogens protein complement may then be compared to the motif and peptides corresponding to the motif may be omitted from the vaccine or may be altered by recombinant means to produce a vaccine free of such peptides. Alternatively, vaccines may be developed with particular populations in mind. For individuals suffering from or at risk of developing a particular autoimmune disease, special vaccines may be developed. In this case, the motif is again

chosen on the basis of the HLA-DR protein associated with the autoimmune disease and, when known, the TCR contact residues of the self epitope.

In particular embodiments, such vaccine preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. And, in particular, vaccines are provided lacking a peptide corresponding to the PV motif #1 disclosed herein. Specific embodiments of the vaccines include vaccines lacking at least one of the peptides disclosed as SEQ ID NO.: 1 through SEQ ID NO.: 7. Thus, methods of immunizing an individual against pathogens which may cause pemphigus vulgaris are also provided.

Similarly, vaccine preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. And, in particular, vaccines lacking a peptide corresponding to at least one of the three MS motifs disclosed herein are provided. Specific embodiments of the vaccines include vaccines lacking at least one of the peptides disclosed as SEQ ID NO.: 8 through SEQ ID NO.: 15. In particularly preferred embodiments, the vaccines are developed from the pathogens listed in Table 2 but lack either the entire protein listed as the antigen or lack at least the peptides identified in the corresponding sequences. Thus, methods of immunizing an individual against pathogens which may cause multiple sclerosis are also provided.

These peptides will also be useful in assessing which pathogen(s) may be important in a particular patient. For example, the T cells from one patient may proliferate in response to one or a few of these peptides, while those from another patient may proliferate in response to a different peptide or set of peptides. Analogs of the peptides may be

synthesized in which one of the T cell receptor contact residues is substituted. For example, in the case of MS, peptides with the substitution of MBP91F by 91A or of MBP93K by 93A may be employed. Such analogs are not, however, limited to the substitution of these primary T cell receptor contact residues or to substitution by particular amino acids such as A. These peptide analogs may be used to anergize (inactive) autoreactive T cells (see, for example, Sloan-Lancaster et al., 1993 and 1994) upon administration to autoimmune patients. The viruses or bacterial pathogens may be useful in immunization by selecting viral or bacterial strains that do not carry the mimicry epitope. Proteins from these pathogens other than those that carry the mimicry epitope may also be selected for immunization. This therapy may be useful in preventing reinfection and thus remissions of the disease or in preventing initial infections in particularly susceptible populations (the most obvious example of which is a disease-free identical twin of a patient).

EXAMPLES

1. Identification of Self Epitopes of Pemphigus Vulgaris

As noted above, pemphigus vulgaris (PV) is, in different ethnic groups, associated either with a DR4 allele (DRB1*0402) or with a rare DQ1 allele (DQB1*05032); only a small fraction of PV patients have neither susceptibility gene (Ahmed et al., 1991; Ahmed et al., 1990; Scharf et al., 1988b). The PV associated molecule has a negative charge (Glu) at the critical position β 71; the neighboring position (β 70) is also negatively charged. The DR4 subtype associated with PV is the only one that carries a negative charge at DR β 71 (a positive charge (Arg) is found at DR β 71 in the RA associated DR4 molecules).

Although polymorphic, the P7 pocket residue DR β 67 (Leu/Ile) does not appear to be involved in peptide binding but probably acts as a TCR contact residue (Stern et al., 1994).

The charge of a polymorphic residue at DR β 71 could therefore account for susceptibility to two different autoimmune syndromes associated with structurally similar DR4 subtypes: DR4 alleles associated with susceptibility to rheumatoid arthritis have a positive charge at DR β 71 (Arg) while the DR4 allele associated with pemphigus vulgaris has a negative charge at DR β 71 (Glu). Peptides selective for either DR4 molecule may therefore differ significantly in their charge at P4: Peptides with a negative charge at P4 would be expected to bind to the RA associated molecules but not the pemphigus associated DR4 molecule; in contrast a positive charge would be expected for the pemphigus peptide(s) at position 4. Due to the conserved nature of these molecules other peptide anchor residues (P1 and P6) would not be expected to be different for these DR4 subtypes.

A sequence motif for selective binding to the HLA-DR DRB1*0402 protein was developed according to the method disclosed herein.

The β chain residues involved in forming the P1 pocket for this allele are β 85 (Val), β 86 (Val), β 89 (Phe) and β 90 (Thr). Thus, the presence of Val at β 86 (instead of Gly as in DRB1*0101) suggests that the P1 position of the motif be restricted to V, L, I, M and F. Alanine might also have been included but was not in this example. The P6 pocket is formed in part by the β 11 (Val) and β 13 (His) of the DRB1*0402 protein. Relative to the DRB1*0101 allele, in which these residues are Leu and Phe respectively, the P6 pocket of the DRB1*0402 protein is somewhat larger and more polar. Thus,

for the P6 position of the motif, S, T, N and V were allowed. Finally, the P4 pocket of this DR protein is formed in part by the residues β 13 (His), β 70 (Asp), β 71 (Glu); β 74 (Ala) and β 78 (Tyr). As noted above, the two negatively charged residues as β 70 and β 71 create a preference for positively charged antigen residues and, therefore, the motif for the P4 position was restricted to K and R.

Thus, the sequence motif for the pemphigus vulgaris autoantigen was defined as:

Position	P1	P2	P3	P4	P5	P6
PV Motif #1:	V	X	X	K	X	S
	L			R		T
	I					N
	M					V
	F					

Although the autoantigen for pemphigus vulgaris is known, the precise epitopes within the autoantigen have previously remained unknown. Using the method of the present invention, however, it has been possible to identify a small set of peptides which may serve as the autoantigenic determinants. The target antigen of pemphigus vulgaris is an epithelial adhesion molecule of the cadherin family, desmoglein 3 (Amagai et al., 1991). Desmoglein 3 mediates Ca^{++} dependent adhesion between keratinocytes; the autoantibodies interfere with cell adhesion with resulting blister formation (Takeichi, 1990). The autoantibodies are thought to be pathogenic since a transient blistering disease is also seen in newborns of affected mothers due to transfer of maternal immunoglobulin to the fetus. Transfer of serum or desmoglein 3 specific antibodies to mice also results in acantholysis (Amagai et al., 1992).

Only seven peptides from this large protein (130 kDa, 999 amino acids) matched the motif. These seven pemphigus vulgaris antigens (PVA.1-PVA.7) are presented below in Table I with the residues corresponding to the MHC binding positions P1, P4 and P6 underlined.

TABLE I

PVA.1 (res. 78-93)	ATQ <u>KITYRIS</u> GVGID
PVA.2 (res. 97-111)	FGIF <u>VVDKNT</u> GDINI
PVA.3 (res. 190-204)	LNS <u>KIAFKIV</u> SQEPA
PVA.4 (res. 206-220)	TPM <u>FLLSRNT</u> GEVRT
PVA.5 (res. 251-265)	CECN <u>IKVKD</u> VNDNFP
PVA.6 (res. 512-526)	SART <u>LNNRYT</u> GPYTF
PVA.7 (res. 762-786)	QSGT <u>MRTRH</u> STGGTN

Therefore, the selective presentation of one or several of these peptides by the PV associated DRB1*0402 molecule to T cells may be critical for initiating autoimmunity in PV. To test this, T cell lines were raised from blood mononuclear cells of two patients with active disease by stimulation with rIL-2 and tested for recognition of the candidate peptides in a proliferation assay. T cell lines from both patients recognized two peptides from the extracellular domain of desmoglein 3 (PVA.3 and PVA.4) that were located close to the major autoantibody recognition site. These T cell lines were HLA-DR restricted as T cell proliferation was blocked by a monoclonal antibody specific for HLA-DR but not by a control antibody. These desmoglein 3 peptides are therefore candidates for the T cell dependent induction of autoimmunity in pemphigus vulgaris.

The motifs of the present invention relating to the HLA-DR proteins can be taken one step further. In a different ethnic group PV is associated with a rare DQ1 subtype (DQB1*05032) that differs from the common DQ1 subtype only at position 57 of the DQ β chain (Sinha, et al., 1988). In the PV associated molecule DQ β 57 is negatively charged (Asp) whereas in the common DQ1 subtype it is not. The same position on the DQ β chain has also been implicated in susceptibility to diabetes. In diabetes, however, the reverse is true: DQ2 and DQ8 molecules associated with susceptibility to diabetes do not have a negative charge at DQ β 57 (Todd et al., 1987).

Based on these observations it becomes clear that two polymorphic positions in the MHC class II β chain (position 71 of DR β and position 57 of DQ β) are critical for selective peptide binding and the development of autoimmunity. Based on the criteria described above, a diabetes linked peptide would be expected to have a negative charge at P9 since such a peptide would only bind to DQ molecules that do not have the same charge at DQ β 57. In contrast, for the DQ1 associated cases of pemphigus, a peptide with a positive charge at P9 may be selective for the disease associated molecule which carries a negative charge at DQ β 57. In the case of DR4 linked autoimmunity, the charge at peptide position 4 confers selectivity to the disease associated DR4 molecule: RA peptides have a negative charge at P4, PV peptides a positive charge at P4. Motifs for selective peptide binding may therefore prove to be tremendously useful in the identification of key epitopes that initiate human autoimmune diseases. This approach is expected to be useful not only for identifying peptides in PV, RA or diabetes but also for other autoimmune diseases where residues critical in peptide binding have been linked to disease susceptibility.

2. Identification of Mimics of the Multiple Sclerosis MBP Autoantigen

Susceptibility to MS is associated with HLA-DR2 (DRA, DRB1*1501, the most common subtype of DR2) (Spielman et al., 1982; Olerup et al., 1989). This MHC class II molecule is believed to play a critical role in the immunopathogenesis of MS by presenting immunodominant self peptides to autoreactive T cells. Following injection of MBP in experimental animals, T cells specific for immunodominant peptides of MBP mediate an inflammatory response in the CNS that can be accompanied by marked demyelination (reviewed in Zamvil and Steinman, 1990). In previous studies two regions of human MBP were found to be immunodominant (residues 84-102 and 143-168) (Ota et al. 1990; Pette et al. 1990; Martin et al. 1990; Wucherpfennig et al. 1994a). Reactivity to the MBP(84-102) peptide was predominantly seen in subjects carrying HLA-DR2. Using L cell transfectants as antigen presenting cells, HLA-DR2b (DRA, DRB1*1501) was found to serve as the restriction element for these MBP(84-102) specific T cell clones.

The MBP(84-102) peptide binds with high affinity to the HLA-DR2b molecule with two hydrophobic residues serving as the anchors P1 (Val 89 of MBP) and P4 (Phe 92 of MBP) (Wucherpfennig et al., 1994a; Vogt et al. 1994). At position P1, Val 89 can be substituted by other aliphatic amino acids (Leu, Ile), as well as by methionine and phenylalanine; alanine is tolerated at this position but reduces the affinity of the peptide for HLA-DR2b. At position P4, all aliphatic and aromatic residues are permitted; again alanine is tolerated but results in a loss of binding affinity.

A mutational analysis of putative TCR contact points confirms that P3 (Phe 91) and P5 (Lys 93) are TCR contacts for the MBP(85-99) specific clones; other residues such as P-1

(Val 88) and P2 (His 90) are important for some clones but not for others. Substitution of P3 (Phe 91) by alanine abolishes TCR recognition for all clones; some clones tolerated conservative substitutions (eg. tyrosine or aliphatic amino acids) while other clones do not. Substitution of P5 (Lys 93) by arginine is tolerated by most T cell clones but more drastic changes frequently result in a partial or complete loss of T cell reactivity. This analysis demonstrates that P2 (His 90), P3 (Phe 91) and P5 (Lys 93) are the primary TCR contact residues for MBP while P1 (Val 89) and P4 (Phe 92) are the primary MHC contact residues for MBP. This analysis also confirms the present teaching that the TCR contact residues of a motif should be highly if not absolutely conserved.

Based on this structural characterization of the immunodominant MBP(85-99) peptide, three sequence motifs were developed to search a protein database for viral and bacterial peptides that matched these requirements. The motifs focused on the core region of the peptide, residues P-1 to P5 (88-93 of the MBP protein), which contained the MHC and TCR contacts common to all clones. In the first motif, aliphatic amino acids were allowed at the first MHC anchor residue P1 while both aliphatic and aromatic residues were permitted at the second MHC anchor P4. For the TCR contacts, the Phe 91 at P3 was absolutely conserved, the Lys 93 at P5 could be substituted only by arginine, while the His 90 at P2 and the Val 88 at P-1 could be substituted by several structurally related amino acids. Thus, the first motif for multiple sclerosis antigens was defined as:

Position	P-1	P1	P2	P3	P4	P5
MS Motif #1:	V	V	H	F	F	K
	L	L	N		Y	R
	I	I	Q		W	
	A	A	F		V	
	M	M			L	
					I	
					A	
					M	

The second motif omitted the Val 88 P-1 as a TCR contact residue (used only by some clones) and permitted also aromatic amino acids at the first MHC anchor P1 (Val 89). This was done since the MBP(85-99) peptide is presented by different HLA-DR2 subtypes: Presentation by DRB1*1501 requires an aliphatic amino acid or phenylalanine at this position while aliphatic and all aromatic residues at this position can serve as anchor for DRB1*1602. This difference, as explained above, relates to the size of the primary pocket for binding this hydrophobic residue and is determined by the Val/Gly dimorphism at DRB86 (Val in *1501 and Gly in *1602) (Busch et al., 1991). Thus, the second motif for multiple sclerosis antigens was defined as:

Position	P1	P2	P3	P4	P5
MS Motif #2:	F	H	F	F	K
	Y	N		Y	R
	W	Q		W	
	V	F		V	
	L			L	
	I			I	
	A			A	
	M			M	

The third sequence motif represented a modification of the TCR contact residues preferred by a subgroup of MBP(85-99) specific clones. For these clones, P5 (Lys 93) was absolutely

conserved while P3 (Phe 91) could be substituted by some aromatic or aliphatic amino acids. The third motif for multiple sclerosis antigens was defined as:

Position	P1	P2	P3	P4	P5
MS Motif #3:	V	H	F	F	K
	L	F	Y	Y	
	I	Y	W	W	
	A	W	V	V	
	M		L	L	
			I	I	

These HLA-DR motifs also matched well with the structural requirements for an HLA-DQ1 restricted clone specific for the MBP(85-99) peptide. This clone required the same minimal peptide segment as DR2 restricted clones (residues 87-97). As in the DR2 restricted clones, P2 (His 90), P3 (Phe 91) and P5 (Lys 93) appeared to be the primary TCR contact residues. Substitution of these hydrophobic positions by aspartic acid greatly diminished the stimulatory capacity of the peptide while substitutions by other hydrophobic amino acids were tolerated. These data suggest that the MBP(85-99) peptide is bound in a similar fashion to HLA-DR2b and to HLA-DQ1 and that the same peptide residues are critical for interaction with the TCR.

These motifs were used as search criteria in a search of protein databases (PIR and SwissProt) using the Genetics Computer Group software (program: findpatterns). More than 600 sequences of viral and bacterial origin were identified that matched these criteria. From this set, sequences were selected based on the following criteria: (1) Viruses known to cause human pathology, (2) Viruses prevalent in the Northern Hemisphere where MS occurs most frequently, (3) Selected bacterial sequences associated with inflammatory CNS disease

(such as Borrelia burgdorferi) and with invasive infections (such as Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa). Not included were most viruses that cause infections in tropical countries, sequences derived from vaccinia virus and a large number of sequences from E. coli (which is part of the normal intestinal flora). When multiple antigenic variants were present, one or several sequences that best fit the motifs were chosen. The selected peptides were synthesized by Pin-Technology on a 1 mg scale (Chiron Mimotopes, San Diego). Seventy peptides were made which fit motifs #1 and #2 and 59 peptides for motif #3.

These peptides were then tested for their ability to activate human MBP(85-99) specific T cell clones that had been previously established from blood T cells of two patients with relapsing-remitting MS (Wucherpfennig et al., 1994a; Wucherpfennig et al., 1994b). Homozygous B cell lines that expressed DR2 (DRB1*1501 or DRB1*1602) or DQ1 were used as antigen presenting cells (APCs) in these T cell proliferation experiments (Wucherpfennig et al., 1994a). As a positive control, all clones were shown to be activated by the MBP(85-99) peptide. Seven clones were tested with the viral/bacterial peptides selected according to the sequence motifs.

Three of the seven clones tested were efficiently activated by several viral/bacterial peptides. The first clone (Hy.1B11) was HLA-DQ1 restricted whereas the other two clones (Hy.2E11 and Hy.1G11) were HLA-DR2 restricted (Wucherpfennig et al., 1994a). Among the 70 peptides selected according to motifs #1 and #2, three mimicry peptides stimulated the DQ1 restricted clone and two peptides stimulated both of the DR2 restricted T cell clones. Among

the group of 59 peptides selected according to motif #3, one peptide was identified for the DQ1 restricted clone while two were identified for the DR2 restricted clones.

Taken together, the DQ1 restricted T cell clone recognized five structurally related peptides: the immunodominant MBP(85-99) peptide, three viral peptides (from Herpes simplex, Adenovirus type 12 and Human Papillomavirus) and a bacterial peptide (Pseudomonas aeruginosa). Two of the DR2 restricted clones were activated by four peptides. Both clones recognized the MBP(85-99) peptide as well as peptides from EBV and Influenza virus. In addition, one clone recognized a viral peptide from Reovirus (clone Hy.2E11) and one recognized a peptide from Herpes simplex virus (clone Hy.1G11). These results, and the sequences of these peptides, are summarized below in Table 2.

TABLE 2

Peptides Recognized by Clone Hy.1B11 (DQ1 Restricted):

MBP(85-99)	ENPVVHFFKNI V TPR
Herpes Simplex, UL15 Protein	FRQLVHFVRDFAQLL
Adenovirus Type 12, ORF	DFEVVTFLKDVLPEF
Pseudomonas aeruginosa, Phosphomannomutase	DRLLMLFAKDVV <u>S</u> RN
Human Papillomavirus Type 7, L2 Protein	IGGRVHFFKDISPIA

Peptides Recognized by Clone Hy.2E11 (DR2 Restricted):

MBP(85-99)	ENPVVHFFKNI V TPR
Epstein-Barr Virus, DNA Polymerase	TGGVYHFVKKH V HES
Influenza Type A, Hemagglutinin	YRN L VWF I KKNTRYP
Reovirus Type 3, Sigma 2 Protein	MARAALFKTVGF G GG

Peptides Recognized by Clone Hy.1G11 (DR2 Restricted):

MBP(85-99)	ENPVVHFFKNIIVTPR
Epstein-Barr Virus, DNA polymerase	TGGVYHFVKKHVHES.
Influenza Type A, Hemagglutinin	YRNLLWIFIKKNTRYP
Herpes Simplex, DNA Polymerase	GGRRRLFFVKAHVRES

For these viral/bacterial peptides to be involved in the initiation of autoimmunity, they have to be capable of potent T cell stimulation that results in marked clonal expansion of autoaggressive T cell clones. The stimulatory capacity of each of these peptides was therefore compared to the MBP(85-99) peptide in a titration experiment. The peptides were found to be efficient stimulators of the MBP specific T cell clones; in particular the EBV peptide (DR2 restricted clones) and the adenovirus peptide (DQ1 restricted clone) were similar to the MBP(85-99) peptide in their stimulatory capacity. These results demonstrate that the T cell activation is not the result of a minor degree of 'cross-reactivity' but rather the result of structural similarity sufficient for potent T cell activation.

Comparison of the peptide sequences that were found to stimulate the same TCR revealed several interesting points:

(1) Only one peptide (Human Papillomavirus L2 protein) had striking sequence similarity with the MBP(85-99) peptide in that all amino acids in the MBP(89-95) segment except position 94 (Asn to Asp) were identical (Table 2). For all other sequences, simple alignment would not have predicted them to be efficient stimulators of MBP(85-99) specific T cell clones. Therefore, absent the method of the present invention, these peptides would not have been identified.

(2) At positions not specified by the search criteria, the selection for particular amino acids was still apparent (Table 2)... For the DQ1 restricted clone, for example; aspartic acid was selected at position P6 (residue 94 of MBP), a probable TCR contact residue, in all four peptides. This position is occupied by asparagine in the MBP peptide (similar size, but no negative charge). Substitution of Asn 94 for Asp in the MBP peptide markedly increased its stimulatory capacity for the DQ1 restricted clone but reduced it for the DR2 restricted clone. Selection also occurred at the neighboring P7 MHC contact (Ile 95) for which Ile, Val or Phe were selected (all hydrophobic).

(3) Different selection events occurred for the DQ1 and the DR2 restricted clones: At position P6 (94 of MBP), there was selection of aspartic acid (negative charge) for DQ1 peptides whereas there was selection for lysine (positive charge) in two of the three peptides presented by DR2.

(4) In the flanking segments (residues 85-87 and 97-99), no apparent selection took place as amino acids with different size and charge were allowed.

The majority of the viruses from which these sequence peptides were identified are common human pathogens: Influenza Type A frequently causes respiratory tract infections; Human Papillomavirus infects epithelial tissues and has been linked to cervical carcinomas; and Epstein-Barr Virus (EBV) causes an acute viral syndrome (infectious mononucleosis) in young adults. Human Herpesvirus I (Herpes simplex), EBV and Human Papillomavirus cause latent or persistent infections with neurons (Herpes simplex), B cells (EBV) and epithelial cells (Papillomavirus) serving as the reservoirs. Viral expression can be reactivated by UV exposure and stress (Herpes simplex)

and by B cell activation (EBV) (Schwarz et al., 1985; Epstein et al., 1977; Spruance 1985; Tovey et al., 1978). For the induction and maintenance of an autoimmune response these persistent viral infections are of particular interest as they could explain the chronicity of the clinical disease and the clonal expansion and persistence of MBP specific T cells. Reactivation of viral expression may also be involved in triggering clinical relapses. By this mechanism, viral peptides could activate resting MBP specific T cells in periphery and allow them to invade the CNS.

Are these foreign epitopes actually presented to autoreactive T cells during a viral infection? The peptide from the EBV DNA polymerase allowed this question to be addressed. In EBV transformed B cells (which were used as antigen presenting cells in the T cell assays) the lytic viral cycle is repressed. The DNA polymerase gene is not transcribed in this latent state; however, B cell activation results in activation of the lytic cycle and in the expression of the DNA polymerase gene (Datta et al., 1980). To examine MHC class II restricted presentation of the EBV DNA polymerase, an HLA-DR2⁺ EBV transformed B cell line (MGAR) and an MHC mismatched control (9001, HLA-DR1) were pretreated for 36 hours with phorbol ester which was removed by extensive washing prior to coculture of APC with T cells. T cell clones Hy.2E11 and Hy.1G11, which recognize the EBV DNA polymerase peptide presented by HLA-DR2, were activated by a HLA-DR2⁺ EBV transformed B cell line pretreated with phorbol ester. This effect was specific because MHC mismatched B cells did not activate the clones; also, a control clone (Ob.1A12) that recognized MBP(85-99) but not the EBV peptide was not activated. In a separate experiment, T cell activation was

blocked by a mAb specific for HLA-DR (mAb L243) but not by a mAb specific for HLA-DQ (G2a.5). These results demonstrate that the MBP specific T cell clones recognize not only the viral peptide but also antigen presenting cells infected with the virus. In vivo this recognition event could lead to chronic antigenic stimulation of MBP specific T cells as B cell activation results in the expression of EBV genes, including the DNA polymerase gene.

Finally, the presentation of the viral peptides by different DR2 subtypes was compared to determine if they are efficiently presented by the disease associated molecule (DRB1*1501, the most common DR2 subtype). The MBP peptide was presented by three of the four DR2 subtypes; the peptide was not presented by DRB1*1601 which differs from DRB1*1602 by a single amino acid substitution (at position DR β 67, a possible TCR contact). The two viral peptides were presented much better by the DR15 molecules (DRB1*1501 and 1502 which differ only at position DR β 86) than by DRB1*1602. This was particularly evident for the influenza peptide which only activated the T cell clone when presented by the DRB1*1501/1502 molecules but not by DRB1*1602. These results indicate that the viral peptides identified herein are preferentially presented by the MS associated DR2 molecule (DRB1*1501).

DEFINITIONS

For clarity of interpretation and to clearly and distinctly point out the subject matter of the claimed invention, the following definitions are provided for several terms used in the claims appended hereto.

The term "sequence motif," in accordance with description provided herein, means a series of restrictions on the residues which may occupy certain relative positions of an amino acid sequence. A sequence motif must restrict at least three and preferably four or five positions of an amino acid sequence. The relative positions of the first (N-terminal) and last (C-terminal) restricted amino acid positions shall be separated by at least two but no more than twelve amino acid residues. For example, P₁ and P₄ may be the first and last restricted residues and these residues are separated by two residues. As another example, P-1 and P₁₁ may be the first and last restricted residues and these are separated by ten residues. Positions between the first and last restricted positions may be restricted or unrestricted with the exception that a total of at least three positions of the motif must be restricted. Of the three positions which must be restricted, at least two must be residues corresponding to major MHC binding pockets. If only two of the restricted residues correspond to MHC binding residues, the third must correspond to a TCR contact residue. Further, at least one of the positions restricted must correspond to either the P₁ or P₄ binding position. By "restricted" is meant that at least one, and preferably ten, amino acid residues shall be excluded from a position.

An amino acid sequence "corresponds" to a sequence motif if it can be aligned with the positions of the sequence motif such that, at each restricted position of the motif, the amino acid sequence includes a residue which is not excluded from that position by the restrictions which define the motif. As the restrictions which define the motif are derived from the size and nature of the MHC binding pockets of HLA-DR proteins

and, optionally, the TCR contact residues of known epitopes, the restricted positions of the binding motif may also be said to correspond to the MHC binding pockets and TCR contact residues.

The term "isolated," in reference to a protein or polypeptide, means separated from its native or natural chemical microenvironment. Thus, a polypeptide isolated from a bacterium shall be in a preparation substantially free of most of the other bacterial polypeptides and, similarly, an isolated viral polypeptide preparation shall be substantially free of the other polypeptides which comprise the virus.

The term "associated with," as used in connection with a particular HLA-DR protein and an autoimmune disease or autoimmune response, shall mean that the protein and the disease/response have been positively correlated by clinical or epidemiological studies such that the likelihood of developing the disease/response has been shown to be increased by presence of the protein.

The term "HLA-DR protein" means the particular protein product of a particular allele of the MHC class II HLA-DR gene. A disease associated with an HLA-DR protein is one associated with such a particular protein and not merely with the HLA-DR gene locus.

By the term "human pathogen" is meant a bacterium, virus or protozoon capable of infecting humans and generating an immune response. The term specifically is intended to exclude the bacteria which form part of the normal human intestinal flora. By this term, "normal human intestinal flora," is meant the bacteria which normally inhabit the human gut, such as Escherichia coli, but which do not normally cause disease.

By the term "autoreactive," as applied to T cells, is meant T cells from a human which are activated by a human self epitope. By "activation" of T cells is meant induction to proliferate, secrete lymphokines (cytokines) and/or to initiate effector activity (e.g. cytotoxicity).

By the term "autoantigen" is meant a self protein or polypeptide which includes a "self epitope." By "self epitope" is meant that part of an autoantigen which is recognized by T cells when bound to and presented by an MHC molecule.

By the term "effective amount," with respect to tolerizing an individual to an antigen, is meant an amount of the antigen sufficient to render T cells, otherwise specific for the antigen, unresponsive to the antigen when bound to and presented by an MHC molecule. T cells which are unresponsive fail to activate when presented with the antigen for which they are specific. By the term "effective amount," with respect to immunizing an individual to an antigen, is meant an amount sufficient to induce an immune response which results in T cells specific for the antigen. Typical ranges of dosages are from 1 nanogram/kilogram to 100 milligrams/kilogram or even 500 milligrams/kilogram. Effective amounts will vary according to such factors as age, sex and sensitivity to the antigen.

By the term "core MHC binding residues" is meant the residues of an epitope corresponding to the P-1 to P9 positions of a peptide bound to an HLA-DR molecule. By the term "inner core MHC binding residues" is meant those residues of an epitope corresponding to the P-1 to P6 positions of a peptide bound to an HLA-DR molecule.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: STROMINGER, JACK L.
WUCHERPENNIG, KAI

(ii) TITLE OF INVENTION: IDENTIFICATION OF SELF AND NON-SELF
ANTIGENS IMPLICATED IN AUTOIMMUNE DISEASE

(iii) NUMBER OF SEQUENCES: 16

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: USA
(F) ZIP: 02210

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: TWOMEY, MICHAEL J.
(B) REGISTRATION NUMBER: 38,349
(C) REFERENCE/DOCKET NUMBER: H0498/7015

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-720-3500
(B) TELEFAX: 617-720-2441

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Thr Gln Lys Ile Thr Tyr Arg Ile Ser Gly Val Gly Ile Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Phe Gly Ile Phe Val Val Asp Lys Asn Thr Gly Asp Ile Asn Ile
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Asn Ser Lys Ile Ala Phe Lys Ile Val Ser Gln Glu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Pro Met Phe Leu Leu Ser Arg Asn Thr Gly Glu Val Arg Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys	Glu	Cys	Asn	Ile	Lys	Val	Lys	Asp	Val	Asn	Asp	Asn	Phe	Pro
1														15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Ala	Arg	Thr	Leu	Asn	Asn	Arg	Tyr	Thr	Gly	Pro	Tyr	Thr	Phe
1														15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Ser Gly Thr Met Arg Thr Arg His Ser Thr Gly Gly Thr Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HERPES SIMPLEX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Arg Gln Leu Val His Phe Val Arg Asp Phe Ala Gln Leu Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: ADENOVIRUS TYPE 12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Phe Glu Val Val Thr Phe Leu Lys Asp Val Leu Pro Glu Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: PSEUDOMONAS AERUGINOSA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Arg Leu Leu Met Leu Phe Ala Lys Asp Val Val Ser Arg Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HUMAN PAPILLOMAVIRUS TYPE 7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Gly Gly Arg Val His Phe Phe Lys Asp Ile Ser Pro Ile Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: EPSTEIN-BARR VIRUS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Thr Gly Gly Val Tyr His Phe Val Lys Lys His Val His Glu Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: INFLUENZA VIRUS TYPE A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Tyr Arg Asn Leu Val Trp Phe Ile Lys Lys Asn Thr Arg Tyr Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: REOVIRUS TYPE 3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ala Arg Ala Ala Phe Leu Phe Lys Thr Val Gly Phe Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HERPES SIMPLEX VIRUS

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Gly Arg Arg Leu Phe Phe Val Lys Ala His Val Arg Glu Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
1 5 10 15

CLAIMS

We claim:

1. An isolated polypeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.
2. An isolated polypeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, and SEQ ID NO.: 15.
3. A pharmaceutical preparation for tolerization comprising a pharmaceutically acceptable carrier and an amount of an isolated human polypeptide effective for tolerizing an individual to an autoantigen, said human polypeptide including an amino acid sequence corresponding to a sequence motif for an HLA-DR protein;
wherein said HLA-DR protein is associated with a human autoimmune disease;
wherein said polypeptide binds to said HLA-DR protein;
wherein said polypeptide bound to said HLA-DR protein activates autoreactive T cells from a subject having said autoimmune disease; and
wherein said polypeptide is a non-collagen and non-myelin basic protein polypeptide.

4. The pharmaceutical preparation of claim 3 wherein said HLA-DR protein is an HLA-DR4 protein and said autoimmune disease is pemphigus vulgaris.

5. The pharmaceutical preparation of claim 4 wherein said motif is PV motif #1.

6. The pharmaceutical preparation of claim 4 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.

7. A pharmaceutical preparation for tolerization comprising a pharmaceutically acceptable carrier and

an amount of an isolated human pathogen polypeptide effective for tolerizing an individual to said polypeptide, said polypeptide including an amino acid sequence corresponding to a sequence motif for an HLA-DR protein;

wherein said HLA-DR protein is associated with a human autoimmune disease;

wherein said polypeptide binds to said HLA-DR protein; and

wherein said polypeptide bound to said HLA-DR protein activates autoreactive T cells from a subject having said autoimmune disease.

8. The pharmaceutical preparation of claim 7 wherein said HLA-DR protein is an HLA-DR2 protein and said autoimmune disease is multiple sclerosis.

9. The pharmaceutical preparation of claim 8 wherein said motif is selected from the group consisting of MS motif #1, MS motif #2 and MS motif #3.

10. The pharmaceutical preparation of claim 8 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, and SEQ ID NO.: 15.

11. A method of tolerizing an individual to an autoantigen of pemphigus vulgaris comprising
administering an effective amount of the pharmaceutical preparation of any one of claims 4-6 to a subject in need of such treatment.

12. A method of tolerizing an individual to a foreign antigen implicated in multiple sclerosis comprising
administering an effective amount of the pharmaceutical preparation of any one of claims 8-10 to an individual in need of such treatment.

13. A pharmaceutical preparation for vaccinating an individual at risk of an autoimmune disease comprising a pharmaceutically acceptable carrier and
an amount of an immunogenic preparation effective to immunize against a human pathogen that in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein;
wherein said HLA-DR protein is associated with said autoimmune disease;

wherein said polypeptide binds to said HLA-DR protein;
wherein said polypeptide bound to said HLA-DR protein
activates autoreactive T cells from a subject having said
autoimmune disease; and

wherein said preparation is free of a polypeptide
corresponding to said sequence.

14. The pharmaceutical preparation of claim 13 wherein said HLA-DR protein is an HLA-DR4 protein and said autoimmune disease is pemphigus vulgaris.

15. The pharmaceutical preparation of claim 14 wherein said motif is PV motif #1.

16. The pharmaceutical preparation of claim 14 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.

17. The pharmaceutical preparation of claim 13 wherein said HLA-DR protein is an HLA-DR2 protein and said autoimmune disease is multiple sclerosis.

18. The pharmaceutical preparation of claim 17 wherein said motif is selected from the group consisting of MS motif #1, MS motif #2 and MS motif #3.

19. The pharmaceutical preparation of claim 17 wherein said amino acid sequence consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, and SEQ ID NO.: 15.

20. A method of vaccinating an individual at risk of pemphigus vulgaris comprising
administering an effective amount of the pharmaceutical preparation of any one of claims 14-16.
21. A method of vaccinating an individual at risk of multiple sclerosis comprising
administering an effective amount of the pharmaceutical preparation of any one of claims 17-19.
22. The pharmaceutical preparation of claim 17 wherein said pathogen and said peptide are selected from the group consisting of the respective pairs Herpes simplex virus and UL15 protein, Herpes simplex virus and SEQ ID NO.: 8, Adenovirus and Adenovirus ORF protein, Adenovirus and SEQ ID NO.: 09, Pseudomonas aeruginosa and phosphomannomutase protein, Pseudomonas aeruginosa and SEQ ID NO.: 10, Papillomavirus and L2 protein, Papillomavirus and SEQ ID NO.: 11, Epstein-Barr virus and DNA polymerase protein, Epstein-Barr virus and SEQ ID NO.: 12, Influenza virus and hemagglutinin protein, Influenza virus and SEQ ID NO.: 13, Reovirus and sigma 2 protein, Reovirus and SEQ ID NO.: 14, Herpes simplex virus and DNA polymerase, and Herpes simplex and SEQ ID NO.: 15.
23. A method of evaluating a peptide for an ability to induce an autoimmune response comprising the steps of
(1) choosing an MHC HLA-DR molecule associated with said autoimmune response, said MHC molecule having major MHC binding pockets designated P1, P4, P6, P7 and P9, each said pocket binding an amino acid residue at a corresponding relative position, Px, of an epitope;

(2) selecting a first major MHC binding pocket P_i and a second major MHC binding pocket P_j;

(3) identifying a first set of amino acid residues which bind within said first pocket, identifying a second set of amino acid residues which bind within said second pocket; and

(4) comparing an amino acid sequence of said peptide to a sequence motif wherein said motif includes said first set of amino acid residues at a relative position P_i and said second set of amino acid residues at a relative position P_j.

24. The method of claim 23

wherein said epitope is an epitope of known amino acid sequence and wherein said epitope has TCR contact residues designated P-1, P2, P3, P5, P8 and P11;

wherein step (2) further comprises selecting a first TCR contact point at a relative position P_k of said epitope and wherein step (3) further comprises identifying a third set of amino acid residues which bind a TCR at said contact point, and

wherein said motif includes said third set of amino acid residues at a relative position P_k.

25. The method of any one of claims 23 and 24 wherein said first binding pocket is a P₁ pocket and said second binding pocket is selected from the group consisting of a P₄ pocket and a P₆ pocket.

26. A method of identifying foreign antigens implicated in human autoimmune response comprising the steps of

(1) choosing an MHC HLA-DR molecule associated with said autoimmune response, said MHC molecule having major MHC binding pockets designated P1, P4, P6, P7 and P9, each said pocket binding an amino acid residue at a relative position Px of an epitope;

(2) selecting a first major MHC binding pocket Pi and a second major MHC binding pocket Pj;

(3) identifying a first set of amino acid residues which bind within said first pocket, identifying a second set of amino acid residues which bind within said second pocket;

(4) defining a sequence motif wherein said motif includes said first set of amino acid residues at a relative position Pi and said second set of amino acid residues at a relative position Pj;

(5) identifying a set of human pathogen peptide sequences corresponding to said motif.

27. The method of claim 26 further comprising the step of excluding from said set sequences from at least one species in a normal human intestinal flora.

28. The method of claim 26 further comprising the step of excluding from said set sequences from at least one species of pathogen negatively correlated with the incidence of said response.

29. The method of claim 26 wherein step (5) includes a search of a computer database using said motif as a search criterion.

ABSTRACT

The present invention provides isolated peptides relating to the autoimmune diseases pemphigus vulgaris and multiple sclerosis. The peptides relating to pemphigus vulgaris are self epitopes and those relating to multiple sclerosis are foreign antigens derived from human pathogens which are implicated in the aetiology and remissions of the disease. Pharmaceutical preparations for tolerizing and/or immunizing individuals are provided as well as methods relating thereto. Methods are provided for identifying other self and non-self epitopes involved in human autoimmune disease and similar pharmaceutical preparations and methods of use for these epitopes are also provided.

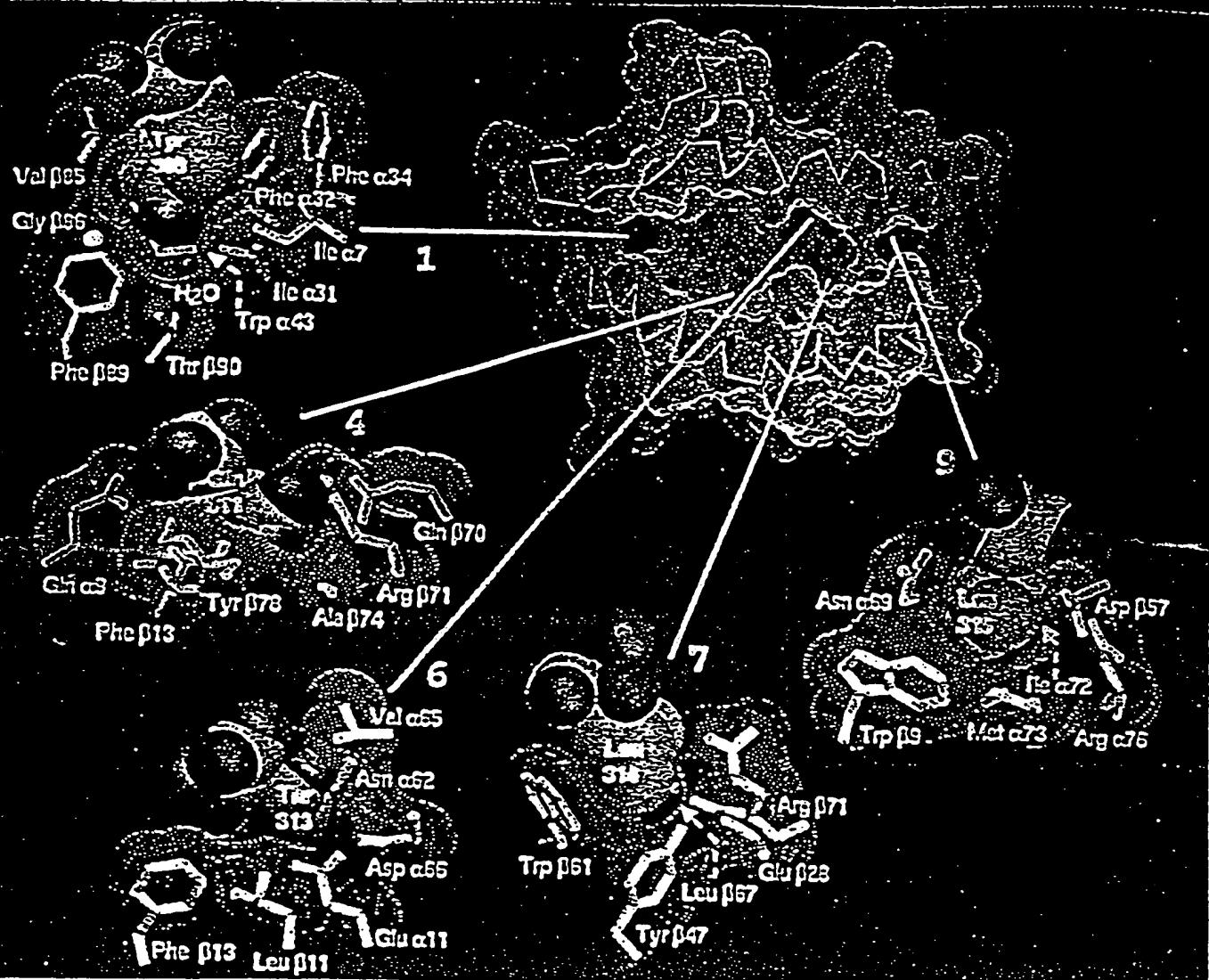


FIGURE 1



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United States Patent [19]
Strominger et al.

[11] Patent Number: **5,874,531**
[45] Date of Patent: **Feb. 23, 1999**

[54] IDENTIFICATION OF SELF AND NON-SELF ANTIGENS IMPLICATED AUTOIMMUNE DISEASE

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[73] Assignee: President and Fellows of Harvard College, Cambridge, Mass.

[21] Appl. No.: 400,796

[22] Filed: Mar. 7, 1995

[51] Int. Cl. A61K 38/04; A61K 39/00

[52] U.S. Cl. 530/326; 424/184.1; 424/185.1; 514/14; 514/21

[58] Field of Search 424/184.1, 185.1; 514/14, 21; 530/326

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Attorney, Agent, or Firm—Testa, Hurwitz & Thibeau

[57]

ABSTRACT

The present invention provides isolated peptides relating to the autoimmune diseases pemphigus vulgaris and multiple sclerosis. The peptides relating to pemphigus vulgaris are self epitopes and those relating to multiple sclerosis are foreign antigens derived from human pathogens which are implicated in the etiology and remissions of the disease. Pharmaceutical preparations for tolerizing and/or immunizing individuals are provided as well as methods relating thereto. Methods are provided for identifying other self and non-self epitopes involved in human autoimmune disease and similar pharmaceutical preparations and methods of use for these epitopes are also provided.

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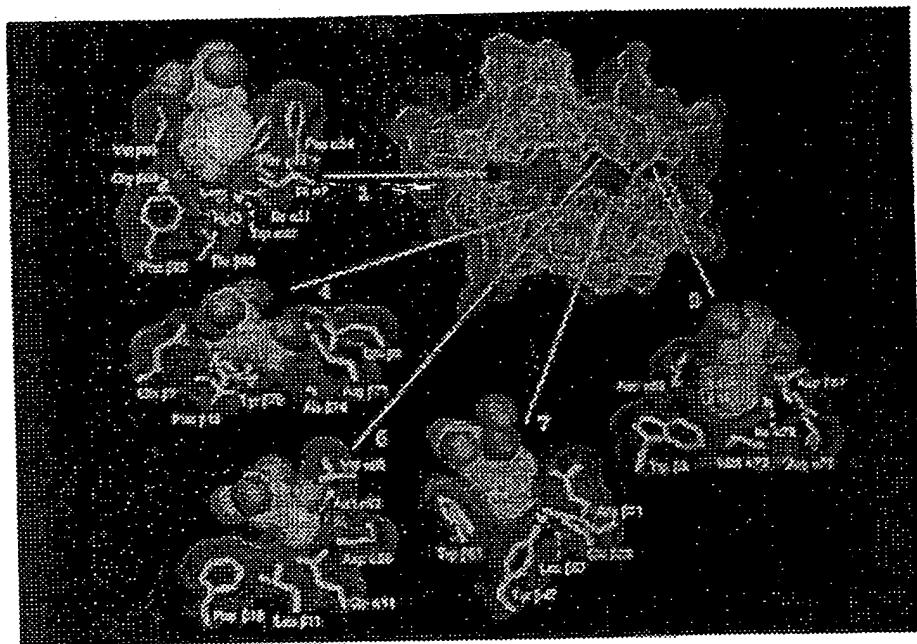


Figure 1

IDENTIFICATION OF SELF AND NON-SELF ANTIGENS IMPLICATED AUTOIMMUNE DISEASE

This invention was supported in part by grant CA47554 from the National Institutes of Health. The government has certain right in the invention.

FIELD OF THE INVENTION

The present invention relates to the field of immunology and, in particular, to the identification of self and non-self antigens implicated in human autoimmune responses. The invention relates to methods of identifying such self and non-self antigens and provides examples of such antigens relating to multiple sclerosis and pemphigus vulgaris. The invention also relates to the use of such antigens for in vitro assays, animal models, therapeutic agents and vaccines.

BACKGROUND OF THE INVENTION

Human autoimmune diseases have a striking genetic association with particular alleles of major histocompatibility complex ("MHC") class I or class II genes. The field was established by the seminal discovery of HLA-B27 linked susceptibility to ankylosing spondylitis, a chronic inflammatory joint disease (Brewerton et al., 1973; Schlossstein et al., 1973). MHC associated susceptibility has now been documented for a variety of human autoimmune diseases, including insulin dependent diabetes mellitus (IDDM), rheumatoid arthritis (RA), pemphigus vulgaris (PV), multiple sclerosis (MS) and myasthenia gravis (MG), just to name a few (Todd et al., 1987; Ahmed et al., 1990; Ahmed et al., 1991; Lanchbury & Panayi, 1991; Spielman & Nathenson, 1982; Protter et al., 1993).

The MHC locus most commonly associated with autoimmune disease is the HLA-DRB locus (also known as DRB1), a highly polymorphic locus with over fifty known alleles. For example, a large body of epidemiological work has documented the association of rheumatoid arthritis with the DR4 (DRB1*0401, DRB1*0404) and DR1 (DRB1*0101) alleles, with the DR4 alleles conferring a higher risk than DR1 (Lanchbury & Panayi, 1991). The risk is dramatically increased when the subject is homozygous or heterozygous for DRB1*0401 and/or DRB1*0404. The observation that arthritis is associated with three DR alleles that are structurally similar led to the development of the 'shared epitope' hypothesis as DRB1*0401, 0404 and 0101 share critical polymorphic residues in the DR β 67-71 cluster (Gregersen et al. 1987; Lanchbury & Panayi, 1991). These residues (in particular DR β 71) appear to be critical in defining the selectivity of peptide binding to the disease associated molecules.

Pemphigus vulgaris is an autoimmune disease of the skin in which high titer autoantibody production to an epidermal cell adhesion molecule (desmoglein 3) results in a loss of keratinocyte adhesion (acantholysis) and severe blister formation (Amagai et al., 1991). In different ethnic groups the disease is associated either with a DR4 allele (DRB1*0402) or with a rare DQ1 allele (DQB1*05032); only a small fraction of PV patients have neither susceptibility gene (Ahmed et al., 1991; Ahmed et al., 1990; Scharf et al., 1988). The DR4 subtype associated with pemphigus differs only at three positions in the DR β 67-71 cluster from the DR4 subtype associated with RA. The PV associated molecule has a negative charge (Glu) at the critical position (DR β 71); the neighboring position (DR β 70) is also negatively charged. The DR4 subtype associated with PV is the only

one that carries a negative charge at DR β 71; a positive charge (Arg) is found at DR β 71 in the RA associated DR4 molecules.

With respect to multiple sclerosis, recent immunological studies suggest that myelin basic protein (MBP) may be one of the important target antigens in the immunopathogenesis of the disease. Several studies have demonstrated that MBP specific T cells are clonally expanded in MS patients and in an in vivo activated state (Allegreita et al., 1990; Wucherpfennig et al., 1994b; Zhang et al., 1994). Reactivity with the immunodominant MBP(84-102) peptide is found predominantly in subjects carrying HLA-DR2 (the most common subtype of which is DRB1*1501), a genetic marker for susceptibility to MS. The MBP(84-102) epitope can also be presented by other MHC class II antigens, including HLA-DQ1 (Ota et al., 1990; Martin et al., 1990; Pette et al., 1990; Wucherpfennig et al., 1994a). In vivo, the T cell response to this peptide appears to be dominated by a few expanded clones.

While associations between MHC alleles and disease states have implicated autoimmunity in the aetiology of these diseases, a large body of clinical and epidemiological evidence suggests that infections may be important in the induction of autoimmunity. For example, particular viral infections frequently precede autoimmune myocarditis and type I diabetes (IDDM) (Rose et al., 1986; Ray et al., 1980). Environmental agents also influence the risk of developing multiple sclerosis as demonstrated by migration studies. Individuals that migrate after age 15 carry the risk for developing MS associated with their geographic origin while individuals who migrate earlier in life acquire the risk of the geographical region to which they migrated (Kurtzke, 1985). These studies are consistent with the hypothesis that a group of pathogens that are relatively ubiquitous in a certain geographic region influence the risk of developing multiple sclerosis (MS). The mechanism(s) leading to clonal expansion of MBP reactive T cells remain to be identified but could involve recognition of viral peptides with sufficient structural similarity to the immunodominant MBP peptide. The initiation of autoimmunity by such a mechanism could then lead to sensitization to other CNS self antigens by determinant spreading (Lehmann et al., 1992; Kaufman et al. 1993; Tisch et al., 1993). Consonant with this hypothesis, it has been noted that inflammatory CNS disease can follow infection with a number of common viral pathogens, such as measles and rubella. On the other hand, the absence of virus in the CNS of these patients and reactivity to myelin basic protein in these patients suggest an autoimmune mechanism (Johnson et al., 1984).

Efforts to identify sequence homologies between self peptide epitopes that might be involved in autoimmunity and various bacterial and viral pathogens have therefore been made. These homology searches have focused on alignments with sequence identity. No success has been reported using such alignments in identifying epitopes from pathogens that could cross react with presumably pathogenic T cell lines from human patients with autoimmune disease (Oldstone, 1990). A sequence identity was recently found between an epitope in a Coxsackie virus protein and GAD65, suspected of being an autoantigen in diabetes. These peptides could reciprocally generate polyclonal T cell lines from mice that cross react with the other peptides (Tian, et al., 1994). No evidence, however, was provided that these peptides could stimulate clones from diabetic mice (or humans).

Recent developments in the field, in particular the identification of allele specific peptide binding motifs have

transformed the field (Madden et al., 1991; Rötschke & Falk, 1991). Based on this knowledge the structural basis for MHC linked susceptibility to autoimmune diseases can be reassessed at a level of detail sufficient for solving long-standing questions in the field. Motifs for peptide binding to several MHC class I and class II molecules have been defined by sequence analysis of naturally processed peptides and by mutational analysis of known epitopes. MHC class I bound peptides were found to be short (generally 8-10 amino acids long) and to possess two dominant MHC anchor residues; MHC class II bound peptides were found to be longer and more heterogeneous in size (Madden et al., 1991; Rötschke & Falk, 1991; Jaretzky et al. 1991, Chicz et al. 1993). Due to the size heterogeneity, however, it has proven more difficult to define MHC class II binding motifs based on sequence alignments. More recently, a crystal structure for HLA-DR1 demonstrated that there is a dominant hydrophobic anchor residue close to the N-terminus of the peptide and that secondary anchor residues are found at several other peptide positions (Brown et al., 1993). Even this work, however, could not provide a detailed description of the binding pockets of HLA-DR proteins, the particular residues involved in the formation of these pockets or the structural requirements or antigens for MHC binding.

In the present disclosure, a detailed description of the HLA-DR antigen binding pockets is provided (Sterk et al., 1994). With this information, together with functional information defining those amino acids of the self or non-self antigen that are needed for MHC binding and TCR contact (e.g., Wucherpfennig et al. 1994a), binding motifs for the various HLA-DR allotypes may be developed, self epitopes involved in autoimmune disease may be identified and a method is provided for identifying bacterial and viral epitopes which may initiate a human autoimmune response.

SUMMARY OF THE INVENTION

The present invention provides, in one aspect, seven different isolated polypeptides derived from the human desmoglein 3 protein and implicated as self epitopes in the autoimmune disease pemphigus vulgaris (PV). These peptides consist essentially of the seven amino acid sequences disclosed herein and designated SEQ ID NO.: 1 through and SEQ ID NO.: 7. In particular, the invention provides isolated peptides which consist of these sequences, the core MHC binding residues of these sequences, or the inner core MHC binding residues of these sequences.

The present invention provides, in another aspect, eight different isolated polypeptides derived from human pathogens but implicated in the aetiology of the autoimmune disease multiple sclerosis. These peptides consist essentially of the eight amino acid sequences disclosed herein and designated SEQ ID NO.: 8 through SEQ ID NO.: 15. In particular, the invention provides isolated peptides which consist of these sequences, the core MHC binding residues of these sequences, or the inner core MHC binding residues of these sequences.

In another set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to autoantigens. The preparations include a pharmaceutically acceptable carrier and an isolated human polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. The peptides are not derived from human collagen or human myelin basic protein.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals having peptides with this motif are provided. Specific embodiments of the pharmaceuticals include each of the polypeptides described above with respect to pemphigus vulgaris. Thus, methods of tolerizing an individual to a pemphigus vulgaris autoantigen are also provided.

In another set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to antigens of human pathogens which are implicated in human autoimmune disease. The preparations include a pharmaceutically acceptable carrier and an isolated human pathogen polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. In addition, three particular sequence motifs are provided for multiple sclerosis and pharmaceuticals having peptides with at least one of these motifs are provided. Specific embodiments of the pharmaceuticals include each of the polypeptides described above with respect to multiple sclerosis. Thus, methods of tolerizing an individual to a multiple sclerosis foreign antigen are also provided.

In another aspect of the invention, pharmaceuticals are provided for vaccination against a human pathogen implicated in the aetiology of autoimmune disease. These pharmaceutical preparations include a pharmaceutically acceptable carrier and an immunogenic preparation effective to immunize against a human pathogen. The human pathogen is one which in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with the autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates T cells which become autoreactive and initiate the autoimmune disease. The preparations of the present invention specifically do not include such polypeptides but, rather, include other antigens from the pathogen.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, a particular sequence motif is provided for pemphigus vulgaris and pharmaceuticals which lack peptides having this motif are provided. Specific embodiments of the pharmaceuticals include preparations lacking each of the polypeptides described above with respect to pemphigus vulgaris. Thus, methods of immunizing an individual against pathogens which may cause pemphigus vulgaris are also provided.

Similarly, pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. Three particular sequence motifs are provided for multiple sclerosis and pharmaceuticals which lack peptides having any of these motifs are provided. Specific embodiments of the pharmaceuticals include preparations lacking each of the polypeptides described above with respect to multiple sclerosis. Thus, methods of immunizing an individual against pathogens which may cause multiple sclerosis are also provided.

The pharmaceutical preparations for immunizing against pathogens that may cause multiple sclerosis may particularly include inactivated forms of the pathogen in which the polypeptide corresponding to the motif are removed or recombinantly altered. Thus, the invention provides such vaccines in which the pathogen and polypeptide are, respectively, Herpes simplex virus and UL15 protein, Herpes simplex virus and SEQ ID NO.: 8, Adenovirus and Adenovirus ORF protein, Adenovirus and SEQ ID NO.: 09, Pseudomonas aeruginosa and phosphomannomutase protein, Pseudomonas aeruginosa and SEQ ID NO.: 10, Papillomavirus and L2 protein, Papillomavirus and SEQ ID NO.: 11, Epstein-Barr virus and DNA polymerase protein, Epstein-Barr virus and SEQ ID NO.: 12, Influenza virus and hemagglutinin protein, Influenza virus and SEQ ID NO.: 13, Reovirus and sigma 2 protein, Reovirus sand SEQ ID NO.: 14, Herpes simplex virus and DNA polymerase, and Herpes simplex and SEQ ID NO.: 15.

The present invention also provides general methods for evaluating a peptide for an ability to induce an autoimmune response. These methods involve choosing an MHC HLA-DR molecule associated with the autoimmune response, selecting at least two major MHC binding pockets of the HLA-DR molecule, identifying sets of amino acid residues which bind within each of the selected pockets, developing a sequence motif for the HLA-DR molecule in which the sets of amino acids define the allowed amino acids at the corresponding positions of the motif, and then comparing the amino acid sequence of the peptide to the sequence motif. Peptides which match the motif have a much greater likelihood of inducing the autoimmune disease. In addition, if there is a known epitope implicated in the disease, the method may further include selecting at least one TCR contact residue of the epitope, identifying a set of amino acid residues which may serve as the TCR contact, and including this set in the motif at the appropriate position. In preferred embodiments, the motifs include restrictions on the residues at positions corresponding to at least the P1 MHC binding pocket and at least one of the P4 and P6 pockets.

In another embodiment of the invention, methods are provided specifically for identifying foreign antigens implicated in human autoimmune response. These methods include the same steps as the previously described methods, but further include a comparison of the resulting sequence motif to sets of human pathogens. In preferred embodiments, peptide sequences from one or more species in the normal human intestinal flora are excluded from consideration. In another preferred embodiment, sequences from one or more species of pathogen which is negatively correlated with the incidence of the disease are excluded. In a most preferred embodiment, the human pathogen peptides are searched and evaluated on a computer database using the motif as a search criterion.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the pockets in the HLA-DR1 peptide binding site. The upper right shows a top view of the molecular surface (1.5 Angstrom probe radius) of the HLA-DR1 peptide-binding site with the C_α trace of the HLA-DR1 shown as thin lines. Pockets that accommodate the peptide side chains of an antigen (in this case Influenza virus hemagglutinin) are shown in detail in surrounding views, and are numbered as described herein. The P1 pocket accommodates the Tyr (308) of the antigen. The P4, P6, P7 and P9 pockets bind antigen residues Gin (311), Thr (313), Leu (314) and Leu (316) respectively. Antigen peptide side chains and the nearby peptide main chain are represented by

CPK models and HLA-DR side chains in contact with the peptide are indicated as stick models. Pockets are viewed in the plane of the peptide binding site, towards the N terminus of the peptide (P1 and P6), towards the C terminus of the peptide (P4), towards the β_1 helical region (P7) or towards the α_1 helical region (P9). A full color version of this figure may be found in Stem et al., 1994.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of identifying and evaluating peptides for their ability to induce an autoimmune response or to cause autoimmune disease. In particular, the invention relates to methods of (1) evaluating self peptides for their potential involvement in autoimmune disease when the self epitope or autoantigen is unknown and (2) evaluating foreign peptides for their possible involvement in the aetiology of autoimmune disease. The invention also relates to specific peptides identified by the methods of the invention and representing self and foreign antigens implicated in, respectively, pemphigus vulgaris and multiple sclerosis.

The methods rely upon the development of amino acid sequence motifs to which potential self or foreign epitopes may be compared. Each motif describes a finite set of amino acid sequences in which the residues at each (relative) position may be (a) restricted to a single residue, (b) allowed to vary amongst a restricted set of residues, or (c) allowed to vary amongst all possible residues. For consistency in this disclosure, but without limiting the invention in any way, these sequence motifs will be symbolized as strings of characters in which (a) a position which is restricted to a single residue will be represented by the one-letter abbreviation for that residue, (b) a position which is allowed to vary amongst a set of residues will be represented by a column of the one-letter abbreviations for those residues, and (c) a position which is allowed to vary amongst all amino acid residues will be represented by an "X." As an example only, a motif might specify that the residue at a first position may be any one of the residues valine, leucine, isoleucine, methionine, or phenylalanine; that the residue at the second position must be histidine; that the residue at the third position may be any amino acid residue; that the residue at the fourth position may be any one of the residues valine, leucine, isoleucine, methionine, phenylalanine, tyrosine or tryptophan; and that the residue at the fifth position must be lysine. Such a motif would be represented by the following character string:

V	H	X	V	K
L			L	
I			I	
M			M	
F			F	
			Y	
				W

In one aspect of the present invention, sequence motifs are developed by analysis of the binding domains or binding pockets of major histocompatibility complex HLA-DR proteins and/or the T cell receptor ("TCR") contact points of epitopes bound to MHC molecules. By providing a detailed structural analysis of the HLA-DR residues involved in forming the MHC binding pockets, one is enabled to make predictions of sequence motifs for binding to any of the HLA-DR proteins.

In another aspect of the present invention, sequence motifs developed by the methods disclosed herein may be

used to identify self peptide epitopes involved in an autoimmune response when the autoantigen is known or suspected.

In another aspect of the present invention, methods of identifying foreign peptide epitopes implicated in autoimmune disease are provided. These methods involve the use of MHC and/or TCR binding motifs to identify peptides derived from certain classes of organisms or pathogens which may initiate human autoimmune response. In this aspect, the motifs may be developed according to the methods of the present invention or by other means known in the art.

Using these sequence motifs as search, evaluation, or design criteria, one is enabled to identify classes of peptides which have a reasonable likelihood of binding to a particular MHC molecule and of interacting with a T cell receptor to induce T cell and/or autoimmune response. Use of these motifs, as opposed to pure sequence homology (which excludes many peptides which are antigenically similar but quite distinct in sequence) or sequence homology with unlimited "conservative" substitutions (which admits many peptides which differ at critical highly conserved sites), represents a significant advance in the ability of one of ordinary skill in the art to evaluate particular peptides for potential involvement in autoimmune disease and to search computer databases of peptide sequences to identify self and foreign peptides which may be involved in autoimmune response. In addition, the use of MHC and/or TCR binding motifs to search limited databases for foreign peptides which may be implicated in the etiology of autoimmune disease is a novel application of the concept of binding motifs.

Detailed examples of the practice of the present invention are presented below. The methods of the invention have now been used to identify the previously unknown self peptide epitopes involved in the autoimmune disease pemphigus vulgaris. In addition, the methods have been used to identify a class of foreign peptides which may be involved in the initiation of autoimmune response in multiple sclerosis (for which the self epitope had previously been identified).

Thus, in another embodiment, the invention provides these peptides, in isolated form, which may be used in various diagnostic and therapeutic methods and products alluded to below.

I. The MHC Class II HLA-DR Molecular Mimicry Motif

The HLA-DR binding site is characterized by five major pockets which may bind the amino acid side chains of antigens (Stern et al., 1994, the entire disclosure of which is incorporated herein by reference). See FIG. 1. The amino acid residue of the antigen which binds in the first major pocket is designated P1. The remaining residues may then be numbered by their positions relative to P1 (with positive numbers increasing toward the carboxy terminus and negative numbers increasing toward the amino terminus):

P-i ... P-1 P1 P2 P3 P4 ... Pj

Thus, the first major pocket of an HLA-DR molecule, by definition, binds the side chain of residue P1 on an antigen. The remaining major pockets bind residues P4, P6, P7 and P9. These residues are defined as the major MHC contact residues.

The amino acid side chains of residues P-1, P2, P3, P5, P8, and P11 are oriented away from the HLA-DR binding site and, therefore, are available as contact residues for a T cell receptor (TCR). All of these residues are defined as TCR contact residues.

A. The MHC Contact Residues

The first major pocket of the HLA-DR molecule is strongly hydrophobic. It is formed by a stretch of residues at

about positions 85, 86, 89 and 90 of the β chain, a stretch of residues at about positions 31, 32 and 34 of the α chain, and side chains from residues at about positions 7 and 43 of the α chain. For example, in HLA-DR1 (DRA, DRB1*0101), the first pocket is formed by residues β 85 (Val), β 86 (Gly), β 89 (Phe), β 90 (Thr), α 31 (Ile), α 32 (Phe), α 34 (Phe), α 7 (Ile), and α 43 (Trp). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992, incorporated by reference herein) and are available through genetic databases.

Although most of the residues that shape the P1 pocket are from the highly conserved DR α chain, the size and nature of this pocket varies due to polymorphisms in the β chain residues involved in the pocket. For the DRB1*0101 protein, the pocket is large and hydrophobic and can accommodate any of the aliphatic or aromatic residues. Polymorphism at the β residues, however, may alter the binding capacity of the P1 pocket. For example, the β 86 residue is known to be polymorphic. Most commonly, this site is occupied by either Gly or Val. Generally, when Gly is present at β 86 (as in DRB1*0101), any of the aliphatic or aromatic residues may bind within the pocket. When Val is present, however, the pocket is smaller and Tyr and Trp cannot be accommodated. Thus, when β 86 is Gly, position P1 of the molecular mimicry motif may consist of residues chosen from V, L, I, A, M, F, Y, W and when β 86 is Val, position P1 of the motif may consist of residues chosen from V, L, I, A, M, F. Similar considerations apply to the other β residues of the P1 pocket.

The P4 pocket of HLA-DR molecules is also a relatively large, shallow, hydrophobic pocket oriented across the antigen binding site. This pocket can bind a variety of large aliphatic side chains which can maintain hydrophobic interactions along the side and floor of the pocket. The pocket is formed by a stretch of residues at about positions 70, 71, 74 and 78 of the β chain, and side chains from residues at about position 13 of the β chain and about position 9 of the α chain. For example, in HLA-DR1 (DRA, DRB1*0101), the P4 pocket is formed by residues β 70 (Gln), β 71 (Arg), β 74 (Ala), β 78 (Tyr), β 13 (Phe), and α 9 (Gln). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

Like the P1 pocket, the P4 pocket is largely hydrophobic but its binding capacity is affected by polymorphisms at the β residues involved in the pocket. For example, different DR alleles have differently charged residues at position β 71: In DRB1*0404, β 71 is occupied by a positively charged Arg residue whereas in DRB1*0402 β 71 is a negatively charged Glu residue. Thus, although this pocket can generally bind a variety of aliphatic or aromatic side chains (e.g., V, L, I, A, M, F, Y, W), positively charged P4 antigen residues are disfavored when β 71 is positively charged and, similarly, negatively charged P4 residues are disfavored when β 71 is also negative. Similar considerations apply to the other β residues of the P4 pocket. Note that some residues may be involved in forming each of two adjacent pockets (e.g., β 13 in the P4 and P6 pockets) and therefore the occupancy of one of these pockets by a particular amino acid may influence the occupancy of the other.

The P6 pocket of HLA-DR molecules is a relatively shallow pocket with a preference for smaller (e.g., A, G) P6 antigen residues. The pocket is formed by the highly conserved α 11, α 62, α 65 and α 66 residues and the highly polymorphic β 11 and β 13 residues of the HLA-DR protein. For example, in HLA-DR1 (DRA, DRB1*0101), the P6 pocket is formed by residues α 11 (Glu), α 62 (Asn), α 65

(Val), α 66 (Asp), β 11 (Leu) and β 13 (Phe). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

Although there are only two β chain residues in the P6 pocket, they vary widely amongst the DR alleles. With a large Phe residue at β 13 (as in DRB1*0101), the P6 residue is preferably one of the small residues (e.g., A, G). In other DR alleles, however, β 13 is occupied by smaller or more polar residues such as the β 13 (His) of DRB1*0401. For such alleles, the P6 motif may include somewhat larger and polar residues (e.g., S, T, V) but should still avoid the largest and aromatic residues. Finally, in some alleles, β 11 and β 13 are both serine residues (e.g., DRB1*1101) and for these cases more hydrophilic or hydrogen bonding residues may be included in the motif.

The P7 pocket of HLA-DR molecules is also a relatively shallow pocket. The pocket is formed by five residues of the β chain: β 28, β 47, β 61, β 67 and β 71. For example, in HLA-DR1 (DRA, DRB1*0101), the P7 pocket is formed by residues β 28 (Glu), β 47 (Tyr), β 61 (Trp), β 67 (Leu) and β 71 (Arg). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases. This pocket does not appear to contribute greatly to the specificity of HLA-DR1 but may be important in other alleles.

The P9 pocket of HLA-DR molecules is generally a small hydrophobic pocket and, therefore, small hydrophobic residues are preferred at the P9 position of the antigen. This pocket is formed by the conserved α chain residues α 69, α 72, α 73 and α 76 and by the polymorphic β chain residues β 9 and β 57. For example, in HLA-DR1 (DRA, DRB1*0101) the P9 pocket is formed by α 69 (Asn), α 72 (Ile), α 73 (Met), α 76 (Arg), β 9 (Trp) and β 57 (Asp). The corresponding residues for other HLA-DR alleles are known in the art (see, e.g., Marsh and Bodmer, 1992) and are available through genetic databases.

The P6, P7 and P9 pockets appear to be less important than the P1 and P4 pockets in binding to DR molecules but they may be more important in binding to other isotypes (e.g., the P9 pocket of DQ may be important).

B. The TCR Contact Residues

When there is no known or suspected antigen involved in an autoimmune response, the positions of the sequence motif corresponding to the TCR contact residues may be left unrestricted. That is, absent a known or suspected antigen, the TCR contact positions of the motif are preferably allowed to vary amongst all of the amino acids.

When, on the other hand, there is a known or suspected antigen involved in an autoimmune response, at least some of the motif positions corresponding to the TCR contact residues may be restricted according to the sequence of the antigen. Thus, for example, the P2 and/or P3 and/or P5 positions of the motif may be restricted to only those residues found at the corresponding positions of the antigen. Alternatively, at least some of the TCR contact residues of the motif may be restricted not just to the corresponding residues of the antigen but may be allowed to vary amongst similarly charged and/or structurally similar residues (e.g., K and R). It should be noted, however, that greater conservatism with respect to the TCR contact residues of the motif is justified by the presumably greater specificity of TCR binding relative to the known promiscuity of MHC binding.

C. Developing an HLA-DR Sequence Motif

Given the present disclosure of the HLA-DR residues involved in the formation of the P1, P4, P6, P7 and P9 MHC binding pockets, and given the nucleotide or corresponding

amino acid sequence of any particular HLA-DR allele, one is now enabled to develop a sequence motif useful in evaluating or predicting the ability of peptides to bind to that MHC protein. When a particular antigen is known to (or is suspected of) binding to the MHC protein, the TCR contact residues of that antigen may also be considered in the motif.

The method first requires the selection of two or more of the MHC binding pockets for which the choice of peptide residues will be restricted at the corresponding positions of the motif. One may select all five of the major binding pockets and develop a motif in which the corresponding five positions of the motif are restricted or one may select fewer and develop a less restricted motif. As will be obvious to one of ordinary skill in the art, a more restricted motif will identify a lesser number of peptides in a database search and a less restricted motif will identify a greater number of peptides. In all instances, at least two of the major binding pockets should be selected. When fewer than all five MHC binding pockets are selected, it is preferred that at least one is P1 and that a second is chosen from P4, P6 and P9.

Either before or after the pockets to be restricted by the motif are selected, the set of amino acid side chains likely to bind within each of those pockets and, therefore, the set of amino acid residues that will define the corresponding positions of the motif, must be determined. This may be accomplished by one of ordinary skill in the art by considering the amino acid residues which form the pocket. These residues, identified in Section A above, will determine the size and nature (i.e., hydrophobic, hydrophilic, positively charged, negatively charged, uncharged) of the pocket and consequently, the side chains which may bind within the pocket. Reference may be had to FIG. 1 during these considerations but will become increasingly unnecessary as one develops familiarity with the variations of the pockets.

As a general matter, in light of the identification of the residues forming the MHC binding pockets of the HLA-DR proteins disclosed herein, one of ordinary skill in the art can easily develop a sequence binding motif for any HLA-DR protein for which these residues are known for two or more binding pockets. The major considerations are size, hydrophobicity and charge. In light of the present disclosure, each of these considerations may be addressed according to well-known principles. A baseline is disclosed herein for each pocket for the DRB1*0101 allele. Relative to this HLA-DR protein, one of ordinary skill is enabled to develop motifs for other HLA-DR alleles. Thus, substitutions which lead to larger/smaller pockets suggest that the corresponding motif positions should be restricted so as to permit smaller/larger residues. Similarly, more/less hydrophobic pockets suggest that the corresponding motif positions should be restricted to more/less hydrophobic residues. Finally, positively/negatively charged pockets suggest that positively/negatively charged residues should be excluded and negatively/positively charged residues may be included at the corresponding motif positions. As noted above, the present disclosure enables one of ordinary skill to develop motifs based upon these well-established principles.

For example, and not by means of limitation, consider the P1 pocket of the HLA-DR protein. The residues forming this pocket in the DRB1*0101 were described above. For DRB1*0101, the P1 pocket is large and hydrophobic and can accommodate any of the aliphatic or aromatic residues (e.g., V, L, I, A, M, F, Y, W). For the DRB1*1602 protein the same is true. On the other hand, in the DRB1*1501 protein, the β 86 position is occupied by Val instead of the Gly found in DRB1*0101 and DRB1*1602. This substitution decreases the size of the P1 pocket in this MHC protein and,

as a result, the pocket cannot easily accommodate Tyr or Trp side chains. Thus, for DRB1*1501, the sequence motif at position P1 may be restricted to residues chosen from V, L, I, A, M and F.

Similarly, in light of the present disclosure, one of ordinary skill in the art may consider each of the MHC binding pockets, or only selected pockets, and develop a sequence motif for any HLA-DR protein for which the residues involved in pocket formation are known. These residues will determine both the size and nature of the pocket and, thereby, the size and nature of the residues which may bind within it. When the pocket is relatively small, the largest amino acid residues (e.g., Y, W) may be excluded from the corresponding position of the motif and, when the pocket is charged, amino acid residues of the same charge may be excluded.

If a self or foreign epitope involved in immune response is known or suspected, and particularly if its TCR contact residues can be defined through the use of responsive T cell clones, the TCR contact residues of the epitope may also be considered in developing a sequence motif. As with the MHC contact residues, all or merely some of the TCR contact residues may be restricted in the motif. And, as with the MHC positions, the restriction of more positions (or the greater restriction of any one position) will result in the identification of fewer peptides in a database search. Unlike the MHC contact residues, for which at least two positions should be restricted in the motif, it is acceptable to omit any restrictions of TCR contact residues in the motif.

If any TCR contact residue positions are restricted in the sequence motif, it is preferred that a position selected from positions P2, P3 and P5 be chosen. Because, in contrast to the relative promiscuity of MHC binding pockets, TCR contact residues appear to have greater specificity, it is preferred that any TCR contact residue positions which are restricted in the motif be rather narrowly restricted. That is, it is preferred that such positions be restricted to just the residue found at the corresponding position of the known antigen or just to residues which are highly similar in structure and charge.

For example, and as further detailed below, the MBP (85-99) peptide is known to be the autoantigen implicated in multiple sclerosis. The P3 residue of this peptide is Phe (residue 91 of MBP) and the P5 residue is Lys (residue 93 of MBP). Thus, if P3 is restricted in the motif, it is preferred that it be restricted to F or, possibly, to F and Y. Similarly, if P5 is restricted, it is preferred that it be restricted to only K or to K and the similarly charged R. Alternatively, P3 and P5 may be left unrestricted.

Obviously, MHC and TCR positions not selected for restriction may be represented by, in the notation of this disclosure, an X. Similarly, as shown in the examples below, several motifs may be developed with varying numbers of positions restricted to varying extents.

II. Identifying Self Epitopes Using Sequence Motifs

An ever increasing number of autoimmune diseases are now being associated with particular alleles of the MHC Class II HLA-DR locus. For most of these autoimmune diseases, the self epitope remains unknown. For some, however, a self protein involved in autoimmune response is known or suspected.

In one aspect of the present invention, a method is provided for identifying the self epitopes involved in autoimmune diseases associated with HLA-DR alleles. That is, by comparing human peptide sequences to the sequence motifs of the present invention, one is enabled to identify those peptides which have the highest likelihood of being the self epitopes involved in the disease.

The method may be applied to any of the autoimmune diseases for which an association with a particular HLA-DR allele is known and for which the amino acid residues forming the MHC binding pockets (or at least two of the major pockets) for that allele are known. In accordance with the method discussed herein, one may then develop one or more sequence motifs for the HLA-DR protein associated with the disease. Naturally, if the disease is associated with two or more alleles, motifs may be developed for the two or more HLA-DR proteins and, in particular, a consensus motif may be developed using the residues shared by each position of these motifs.

The sequence motif or motifs thus developed are then compared to appropriate sets of human peptide sequences.

15 The human peptide sequences may include all known human sequences or may be limited in ways which will be obvious to one of ordinary skill in the art. For example, if the disease is restricted to particular tissues, the search may be limited to peptides found in those tissues. Conversely, peptides 20 which are also found in unaffected tissues may be eliminated from the search pool. In the most extreme case, when the autoantigen is known or suspected but the particular epitope is unknown, the search may be limited to sequences within the autoantigen (see Example 1).

25 This method may be used to identify a set of peptides which match the motif and which are most likely to be self epitopes. By varying the number of positions restricted by the motifs, and/or the extent of restriction at each position, and/or the size of the search pool, the number of peptides in the set will, in all likelihood, also be varied. As noted above, 30 at least two of the MHC contact positions (e.g., P1 and P4) should be restricted. Depending upon the number of peptides in the resultant set, a more or less restrictive motif may then be employed to reduce or expand the set. The desired size of the resultant set depends, of course, upon the subsequent intentions of the practitioner of this method.

Once a set of peptides has been identified, these peptides 35 may optionally be screened for activity. The choice of such screens is at the discretion of the practitioner and beyond the scope of the present invention. Preferred screens, however, 40 include in vitro tests for the ability to induce the proliferation of autoreactive T cells or to induce the secretion of lymphokines (cytokines) from these T cells or to induce other effector functions such as cytotoxicity. In some 45 circumstances, human in vivo tests may be appropriate and in other circumstances animal models of the human disease may be available.

III. Identifying Foreign Epitopes Implicated in Human Autoimmune Disease

As noted in the Background section, epidemiological 50 evidence has suggested that various bacterial and viral pathogens may be implicated in human autoimmune diseases and the concept of molecular mimicry pervades the literature (reviewed by Oldstone, 1990). Prior attempts to identify particular foreign epitopes involved in human 55 autoimmune disease, however, have depended upon direct sequence similarity to known human epitopes. The results have been disappointing and, to date, no pathogens or peptides derived from pathogens have been shown to be a primary cause of human autoimmune disease.

Thus, in another aspect of the present invention, a method 60 of identifying foreign epitopes implicated in human autoimmune disease is provided. That is, for the first time a method of identifying such foreign epitopes is provided which employs sequence motifs to identify foreign peptides which have the highest likelihood of being involved in the etiology of human autoimmune disease.

The method may be applied to any of the autoimmune diseases for which an association with a particular MHC protein is known and for which either (1) a sequence motif has been developed by prior art methods or (2) a sequence motif may be developed by the methods of the present invention. When the self epitope is known or suspected, TCR contact residues may be included in the motif. As before, one or more motifs may be employed and differently derived motifs may be combined to develop consensus motifs.

The sequence motif or motifs thus developed may then be compared to appropriate sets of peptide sequences derived from human pathogens. This is most conveniently accomplished using genetic databases widely available to those of skill in the art. In a most preferred embodiment, the search pool is limited in one or more of the following ways: (1) only sequences from human bacterial or viral pathogens are included; (2) sequences from the normal human intestinal flora (e.g., *E. coli* or other *Enterobacteriaceae*) are excluded; and (3) sequences from pathogens are included/excluded depending upon whether the geographical or epidemiological incidence of the pathogens are positively/negatively correlated with the incidence of the autoimmune disease in question (see Example 2).

This method may be used to identify a set of foreign peptides which match the motif and which are most likely to be involved in the human disease. As before, the number of peptides in the set can be varied by using more or less restrictive motifs and/or by varying the search pool. And, as before, the resultant set of peptides may subsequently be subjected to any of a variety of known screens for activity.

IV. Self and Foreign Epitopes Identified by the Methods of the Present Invention

As detailed in the examples below, the methods of the present invention have been employed (1) to identify seven self epitopes of the desmoglein 3 protein implicated in pemphigus vulgaris and (2) to identify eight foreign epitopes from human pathogens implicated in multiple sclerosis.

Each of these peptides is fifteen residues in length, partly as a result of the computer database search program used (Genetics Computer Group program "Findpatterns") but also corresponding to the size of the cleft in MHC class II molecules. The fifth position of each corresponds to the P1 residue of the antigen. Thus, the P-2 to P11 residues which span the MHC Class II binding cleft correspond to the third through fifteenth residues of these sequences. The P-1 to P9 residues which are important to MHC and TCR binding correspond to the fourth through thirteenth positions. The most important residues for MHC and TCR binding, P-1 to P6 correspond to the fourth through tenth positions of these sequences.

SEQ ID NO.: 1 through SEQ ID NO.: 7, also shown in Table 1 as PVA 1 through PVA 7, correspond to residues 78-93, 97-111, 190-204, 206-220, 251-265, 512-526 and 762-786 of the human desmoglein 3 protein. These peptides are implicated as self epitopes in pemphigus vulgaris. Already, as described in Example 1, two of these peptides have been shown to cause proliferation of T cells isolated from two patients with pemphigus.

SEQ ID NO.: 8 through SEQ ID NO.: 15, also shown in Table 2 are internal fragments of the herpes simplex virus UL15 protein, adenovirus Type 12 ORF, *Pseudomonas aeruginosa* phosphomannomutase, human Papillomavirus Type 7 L2 protein, Epstein-Barr virus DNA polymerase, Influenza Type A hemagglutinin protein, Reovirus Type 3 sigma 2 protein, and herpes simplex DNA polymerase, respectively. These peptides are implicated as foreign

epitopes involved in the aetiology or in remissions of multiple sclerosis. As detailed in Example 2 below, each has been shown to be capable of inducing the proliferation of autoreactive T cell clones isolated from human multiple sclerosis patients. The sequence of the MBP(85-99) peptide is disclosed as SEQ ID NO.: 16.

Each of these proteins has a variety of utilities and, therefore, in another aspect, the present invention provides each of these peptides in isolated form. In addition to the sequences of fifteen residues shown in the Sequence Listing and Tables, the present invention also embraces the fragments of these peptides corresponding to the MHC binding domain. In particular, the invention provides peptides corresponding to the P-2 to P11, P-1 to P9 and P-1 to P6 positions of each of SEQ ID NO.: 1 through SEQ ID NO.: 15. As will be obvious to one of ordinary skill in the art, however, any fragment of any of these peptides which includes at least the P1 and P4 or at least the P1 and P6 or at least the P4, P6 and P7 residues may have utility and is intended to fall within the spirit and scope of the claims. In particular, longer peptides including these peptides or peptides including at least the MHC binding and TCR contact residues described above are contemplated as equivalents.

The manner of production of these peptides is inconsequential but they may be isolated and purified from their natural sources or they may be synthesized. Because of their relatively short length, it is presently contemplated that they should be produced by synthesis. Methods of isolation, purification and synthesis of such peptides are well known in the art and need not be recited herein.

The peptides of the present invention may be used for in vitro assays to aid in the diagnosis and classification of pemphigus vulgaris and multiple sclerosis. For example, autoreactive T cells from patients with PV and MS may be tested, as in the examples below or by other known assays, for reactivity with these peptides. The ability or inability of these peptides to cause proliferation of the T cells will, in the case of pemphigus, allow for a refinement of the diagnosis by particular desmoglein 3 epitopes and, in the case of multiple sclerosis, allow further classification of the disease by cross-reactive (self and foreign epitope) types. Immune response to these peptides prior to onset of disease may also be used as an indication of susceptibility or predisposition although care should be taken so as not to induce autoantigenic response.

The present invention also provides products and methods using other peptides which may be identified by the methods of the present invention. These peptides, as well as those disclosed above, may be used in each of the following embodiments.

The peptides of the present invention may be used in the development of animal models by immunizing animals (e.g., mice, rabbits, non-human primates) with these peptides. Animals which not only develop a response to the peptide but which also develop an autoimmune disease corresponding to the human pathology will have obvious utility as models for the human disease. Animals which develop a response to the peptide without developing any corresponding autoimmune disease, will have utility as subjects for experiments involving the selective depletion of T cells or other forms of desensitization or tolerization.

Importantly, these peptides and amino acid analogs of these peptides will have utility as therapeutic and diagnostic agents. The pathogens, viruses or bacteria from which they are derived will have utility as vaccinating agents. Some examples of the utility of these materials include the following.

The peptide may be administered in high doses to produce high dose tolerance. This process of tolerization is described in, for example, PCT patent application US93/08456 (International publication number WO 94/06828). Thus, in one set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to autoantigens. The preparations may include a pharmaceutically acceptable carrier and an isolated human polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. By using the peptides disclosed herein or identified by the methods of the invention, such pharmaceuticals can be used to combat autoimmune response. The use of such tolerization to human autoimmune diseases is known in the art and need not be elaborated upon here. Tolerizing doses of collagen for rheumatoid arthritis and myelin basic protein for multiple sclerosis have been used. The present invention therefore specifically does not embrace these proteins. Other peptides, however, may now be identified by the present methods and be similarly used to treat autoimmune disease.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. In addition, using PV motif #1, pharmaceuticals having peptides with this motif are provided. In most preferred embodiments, the pharmaceuticals include at least one of the polypeptides of SEQ ID NO.: 1 through SEQ ID NO.: 7. Thus, methods of tolerizing an individual to a pemphigus vulgaris autoantigen are also provided.

In a similar set of embodiments, the invention provides for pharmaceutical preparations for use in tolerizing individuals to antigens of human pathogens which are implicated in human autoimmune disease. The preparations include a pharmaceutically acceptable carrier and an isolated human pathogen polypeptide which includes an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with a human autoimmune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates autoreactive T cells in subjects having the autoimmune disease. Thus, by tolerizing an individual to these antigens, T cells which are cross-reactive with the self antigen will be rendered unresponsive or anergized and protection from the disease will be afforded.

In particular embodiments, such pharmaceutical preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. In addition, using the three MS motifs disclosed herein, pharmaceuticals having peptides with at least one of these motifs are provided. Specific embodiments of the pharmaceuticals include at least one of the polypeptides disclosed as SEQ ID NO.: 8 through SEQ ID NO.: 15. Thus, methods of tolerizing an individual to a multiple sclerosis foreign antigen are also provided.

In another set of embodiments, pharmaceuticals are provided for vaccination against a human pathogen implicated in the aetiology of autoimmune disease. These pharmaceutical preparations include a pharmaceutically acceptable carrier and an immunogenic preparation effective to immunize against a human pathogen. The human pathogen is one which in its native form includes a polypeptide having an amino acid sequence corresponding to a sequence motif for an HLA-DR protein which is associated with the autoim-

mune disease. These polypeptides are capable of binding to the HLA-DR protein to form a complex which activates T cells which become autoreactive and initiate the autoimmune disease. The preparations of the present invention specifically do not include such polypeptides but, rather, include other antigens from the pathogen. That is, a vaccine is produced which specifically does not include polypeptides which correspond to the sequence motifs for the HLA-DR protein and, if known, the TCR contacts of the self epitope. Because pathogens present a wide array of antigenic determinants, one may eliminate those which correspond to the relevant sequence motif and produce a vaccine which is effective against the pathogen but which will not include peptides implicated in the autoimmune response.

Such vaccines, lacking peptides corresponding to the sequence motifs of the present invention, may be made in any convenient manner by one of ordinary skill in the art. For example, when producing an influenza vaccine, one may compare the peptide sequences of the influenza virus to sequence motifs developed according to the present invention. The vaccine may then be made excluding the proteins which have the motif sequences (e.g. by using a fragment of the virus) or recombinant techniques may be used to produce a virus in which the sequences corresponding to the motif are altered such that they do not match the motif. In preferred embodiments, the altered residues are the TCR contact residues and, in particular, a substitution which alters the charge of a TCR contact residue is preferred. Similar vaccines can be developed for bacterial pathogens using, for example, only portions of the bacterium (e.g. bacterial surface proteins or membrane associated proteins) which lack a motif associated with human autoimmune disease or, again, by genetically altering the vaccine bacteria so as to change residues.

The motifs which are considered in creating such vaccines may be chosen on any of several grounds. If the pathogen to which the vaccine is directed is associated with an autoimmune disease, a motif may be developed according to the methods described herein for an HLA-DR protein associated with the disease. If there is also a known or suspected autoantigen, the motif may include TCR contact residues of the self epitope. The pathogen's protein complement may then be compared to the motif and peptides corresponding to the motif may be omitted from the vaccine or may be altered by recombinant means to produce a vaccine free of such peptides. Alternatively, vaccines may be developed with particular populations in mind. For individuals suffering from or at risk of developing a particular autoimmune disease, special vaccines may be developed. In this case, the motif is again chosen on the basis of the HLA-DR protein associated with the autoimmune disease and, when known, the TCR contact residues of the self epitope.

In particular embodiments, such vaccine preparations are provided in which the HLA-DR protein is HLA-DR4 protein and the autoimmune disease is pemphigus vulgaris. And, in particular, vaccines are provided lacking a peptide corresponding to the PV motif #1 disclosed herein. Specific embodiments of the vaccines include vaccines lacking at least one of the peptides disclosed as SEQ ID NO.: 1 through SEQ ID NO.: 7. Thus, methods of immunizing an individual against pathogens which may cause pemphigus vulgaris are also provided.

Similarly, vaccine preparations are provided in which the HLA-DR protein is HLA-DR2 protein and the autoimmune disease is multiple sclerosis. And, in particular, vaccines lacking a peptide corresponding to at least one of the three MS motifs disclosed herein are provided. Specific embodi-

ments of the vaccines include vaccines lacking at least one of the peptides disclosed as SEQ ID NO.: 8 through SEQ ID NO.: 15. In particularly preferred embodiments, the vaccines are developed from the pathogens listed in Table 2 but lack either the entire protein listed as the antigen or lack at least the peptides identified in the corresponding sequences. Thus, methods of immunizing an individual against pathogens which may cause multiple sclerosis are also provided.

These peptides will also be useful in assessing which pathogen(s) may be important in a particular patient. For example, the T cells from one patient may proliferate in response to one or a few of these peptides, while those from another patient may proliferate in response to a different peptide or set of peptides. Analogs of the peptides may be synthesized in which one of the T cell receptor contact residues is substituted. For example, in the case of MS, peptides with the substitution of MBP91F by 91A or of MBP93K by 93A may be employed. Such analogs are not, however, limited to the substitution of these primary T cell receptor contact residues of to substitution by particular amino acids such as A. These peptide analogs may be used to anergize (inactivate) autoreactive T cells (see, for example, Sloan-Lancaster et al., 1993 and 1994) upon administration to autoimmune patients. The viruses or bacterial pathogens may be useful in immunization by selecting viral or bacterial strains that do not carry the mimicry epitope. Proteins from these pathogens other than those that carry the mimicry epitope may also be selected for immunization. This therapy may be useful in preventing reinfection and thus remissions of the disease or in preventing initial infections in particularly susceptible populations (the most obvious example of which is a disease-free identical twin of a patient).

EXAMPLES

1. Identification of Self Epitopes of Pemphigus Vulgaris

As noted above, pemphigus vulgaris (PV) is, in different ethnic groups, associated either with a DR4 allele (DRB1*0402) or with a rare DQ1 allele (DQB1*05032); only a small fraction of PV patients have neither susceptibility gene (Ahmed et al., 1991; Ahmed et al., 1990; Scharf et al., 1988). The PV associated molecule has a negative charge (Glu) at the critical position β 71; the neighboring position (β 70) is also negatively charged. The DR4 subtype associated with PV is the only one that carries negative charge at DR β 71 (a positive charge (Arg) is found at DR β 71 in the RA associated DR4 molecules). Although polymorphic, the P7 pocket residue DR β 67 (Leu/Ile) does not appear to be involved in peptide binding but probably acts as a TCR contact residue (Stern et al., 1994).

The charge of a polymorphic residue at DR β 71 could therefore account for susceptibility to two different autoimmune syndromes associated with structurally similar DR4 subtypes: DR4 alleles associated with susceptibility to rheumatoid arthritis have a positive charge at DR β 71 (Arg) while the DR4 allele associated with pemphigus vulgaris has a negative charge at DR β 71 (Glu). Peptides selective for either DR4 molecule may therefore differ significantly in their charge at P4: Peptides with a negative charge at P4 would be expected to bind to the RA associated molecules but not the pemphigus associated DR4 molecule; in contrast, a positive charge would be expected for the pemphigus peptide(s) at position 4. Due to the conserved nature of these molecules, other peptide anchor residues (P1 and P6) would not be expected to be different for these DR4 subtypes.

A sequence motif for selective binding to the HLA-DR DRB1*0402 protein was developed according to the method disclosed herein.

The β chain residues involved in forming the P1 pocket for this allele are β 85 (Val), β 86 (Val), β 89 (Phe) and β 90 (Thr). Thus, the presence of Val at β 86 (instead of Gly as in DRB1*0101) suggests that the P1 position of the motif be restricted to V, L, I, M and F. Alanine might also have been included but was not in this example. The P6 pocket is formed in part by the β 11 (Val) and β 13 (His) of the DRB1*0402 protein. Relative to the DRB1*0101 allele, in which these residues are Leu and Phe respectively, the P6 pocket of the DRB1*0402 protein is somewhat larger and more polar. Thus, for the P6 position of the motif, S, T, N and V were allowed. Finally, the P4 pocket of this DR protein is formed in part by the residues β 13 (His), β 70 (Asp), β 71 (Glu), β 74 (Ala) and β 78 (Tyr). As noted above, the two negatively charged residues as β 70 and β 71 create a preference for positively charged antigen residues and, therefore, the motif for the P4 position was restricted to K and R.

Thus, the sequence motif for the pemphigus vulgaris autoantigen was defined as:

Position	P1	P2	P3	P4	P5	P6
PV Motif #1:	V	X	X	K	X	S
	L			R		T
	I					N
	M					V
	F					

Although the autoantigen for pemphigus vulgaris is known, the precise epitopes within the autoantigen have previously remained unknown. Using the method of the present invention, however, it has been possible to identify a small set of peptides which may serve as the autoantigenic determinants. The target antigen of pemphigus vulgaris is an epithelial adhesion molecule of the cadherin family, desmoglein 3 (Amagai et al., 1991). Desmoglein 3 mediates Ca^{++} dependent adhesion between keratinocytes; the autoantibodies interfere with cell adhesion with resulting blister formation (Takeichi, 1990). The autoantibodies are thought to be pathogenic since a transient blistering disease is also seen in newborns of affected mothers due to transfer of maternal immunoglobulin to the fetus. Transfer of serum or desmoglein 3 specific antibodies to mice also results in acantholysis (Amagai et al., 1992).

Only seven peptides from this large protein (130 kDa, 999 amino acids) matched the motif. These seven pemphigus vulgaris antigens (PVA.1-PVA.7) are presented below in Table 1 with the residues corresponding to the MHC binding positions P1, P4 and P6 underlined.

TABLE 1

PVA.1 (res. 78-93)	ATQKITY <u>R</u> ISGVVGID
PVA.2 (res. 97-111)	FGIF <u>V</u> VD <u>K</u> NTGDINI
PVA.3 (res. 190-204)	LNSKIA <u>F</u> KVVSQEP <u>A</u>
PVA.4 (res. 206-220)	TPMF <u>L</u> LSRNTGEV <u>R</u> T
PVA.5 (res. 251-265)	CECN <u>I</u> KV <u>D</u> VNDN <u>F</u> P
PVA.6 (res. 512-526)	SART <u>L</u> NN <u>R</u> Y <u>T</u> G <u>P</u> YTF
PVA.7 (res. 762-786)	QSGT <u>M</u> RTR <u>H</u> STGGTN

Therefore, the selective presentation of one or several of these peptides by the PV associated DRB1*0402 molecule to T cells may be critical for initiating autoimmunity in PV. To test this, T cell lines were raised from blood mononuclear cells of two patients with active disease by stimulation with the seven candidate peptides. T cell lines were expanded with rIL-2 and tested for recognition of the candidate peptides in a proliferation assay. T cell lines from both patients recognized two peptides from the extracellular

domain of desmoglein 3 (PVA.3 and PVA.4) that were located close to the major autoantibody recognition site. These T cell lines were HLA-DR restricted as T cell proliferation was blocked by a monoclonal antibody specific for HLA-DR but not by a control antibody. These desmoglein 3 peptides are therefore candidates for the T cell dependent induction of autoimmunity in pemphigus vulgaris.

The motifs of the present invention relating to the HLA-DR proteins can be taken one step further. In a different ethnic group PV is associated with a rare DQ1 subtype (DQB1*05032) that differs from the common DQ1 subtype only at position 57 of the DQ β chain (Sinha, et al., 1988). In the PV associated molecule DQ β 57 is negatively charged (Asp) whereas in the common DQ1 subtype it is not. The same position on the DQ β chain has also been implicated in susceptibility to diabetes. In diabetes, however, the reverse is true: DQ2 and DQ8 molecules associated with susceptibility to diabetes do not have a negative charge at DQ β 57 (Todd et al., 1987).

Based on these observations it becomes clear that two polymorphic positions in the MHC class II β chain (position 71 of DR β and position 57 of DQ β) are critical for selective peptide binding and the development of autoimmunity. Based on the criteria described above, a diabetes linked peptide would be expected to have a negative charge at P9 since such a peptide would only bind to DQ molecules that do not have the same charge at DQ β 57. In contrast, for the DQ1 associated cases of pemphigus, a peptide with a positive charge at P9 may be selective for the disease associated molecule which carries a negative charge at DQ β 57. In the case of DR4 linked autoimmunity, the charge at peptide position 4 confers selectivity to the disease associated DR4 molecule: RA peptides have a negative charge at P4, PV peptides a positive charge at P4. Motifs for selective peptide binding may therefore prove to be tremendously useful in the identification of key epitopes that initiate human autoimmune diseases. This approach is expected to be useful not only for identifying peptides in PV, RA or diabetes but also for other autoimmune diseases where residues critical in peptide binding have been linked to disease susceptibility.

2. Identification of Mimics of the Multiple Sclerosis MBP Autoantigen

Susceptibility to MS is associated with HLA-DR2 (DRA, DRB1*1501, the most common subtype of DR2) (Spielman et al., 1982; Olerup et al., 1989). This MHC class II molecule is believed to play a critical role in the immunopathogenesis of MS by presenting immunodominant self peptides to autoreactive T cells. Following injection of MBP in experimental animals, T cells specific for immunodominant peptides of MBP mediate an inflammatory response in the CNS that can be accompanied by marked demyelination (reviewed in Zamvil and Steinman, 1990). In previous studies two regions of human MBP were found to be immunodominant (residues 84-102 and 143-168) (Ota et al. 1990; Pette et al. 1990; Martin et al. 1990; Wucherpfennig et al. 1994a). Reactivity to the MBP(84-102) peptide was predominantly seen in subjects carrying HLA-DR2. Using L cell transfectants as antigen presenting cells, HLA-DR2b (DRA, DRB1*1501) was found to serve as the restriction element for these MBP(84-102) specific T cell clones.

The MBP(84-102) peptide binds with high affinity to the HLA-DR2b molecule with two hydrophobic residues serving as the anchors P1 (Val 89 of MBP) and P4 (Phe 92 of MBP) (Wucherpfennig et al., 1994a; Vogt et al. 1994). At position P1, Val 89 can be substituted by other aliphatic amino acids (Leu, Ile), as well as by methionine and phenylalanine; alanine is tolerated at this position but

reduces the affinity of the peptide for HLA-DR2b. At position P4, all aliphatic and aromatic residues are permitted; again alanine is tolerated but results in a loss of binding affinity.

A mutational analysis of putative TCR contact points confirms that P3 (Phe 91) and P5 (Lys 93) are TCR contacts for the MBP(85-99) specific clones; other residues such as P-1 (Val 88) and P2 (His 90) are important for some clones but not for others. Substitution of P3 (Phe 91) by alanine abolishes TCR recognition for all clones; some clones tolerated conservative substitutions (eg. tyrosine or aliphatic amino acids) while other clones do not. Substitution of P5 (Lys 93) by arginine is tolerated by most T cell clones but more drastic changes frequently result in a partial or complete loss of T cell reactivity. This analysis demonstrates that P2 (His 90), P3 (Phe 91) and P5 (Lys 93) are the primary TCR contact residues for MBP while P1 (Val 89) and P4 (Phe 92) are the primary MHC contact residues for MBP. This analysis also confirms the present teaching that the TCR contact residues of a motif should be highly if not absolutely conserved.

Based on this structural characterization of the immunodominant MBP(85-99) peptide, three sequence motifs were developed to search a protein database for viral and bacterial peptides that matched these requirements. The motifs focused on the core region of the peptide, residues P-1 to P5 (88-93 of the MBP protein), which contained the MHC and TCR contacts common to all clones. In the first motif, aliphatic amino acids were allowed at the first MHC anchor residue P1 while both aliphatic and aromatic residues were permitted at the second MHC anchor P4. For the TCR contacts, the Phe 91 at P3 was absolutely conserved, the Lys 93 at P5 could be substituted only by arginine, while the His 90 at P2 and the Val 88 at P-1 could be substituted by several structurally related amino acids. Thus, the first motif for multiple sclerosis antigens was defined as:

Position	P-1	P1	P2	P3	P4	P5
MS Motif #1:	V	V	H	F	F	K
	L	L	N		Y	R
	I	I	Q		W	
	A	A	F		V	
	M	M			L	
					I	
					A	
					M	

The second motif omitted the Val 88 P-1 as a TCR contact residue (used only by some clones) and permitted also aromatic amino acids at the first MHC anchor P1 (Val 89). This was done since the MBP(85-99) peptide is presented by different HLA-DR2 subtypes: Presentation by DRB1*1501 requires an aliphatic amino acid or phenylalanine at this position while aliphatic and all aromatic residues at this position can serve as anchor for DRB1*1602. This difference, as explained above, relates to the size of the primary pocket for binding this hydrophobic residue and is determined by the Val/Gly dimorphism at DR β 86 (Val in *1501 and Gly in *1602) (Busch et al., 1991). Thus, the second motif for multiple sclerosis antigens was defined as:

Position	P1	P2	P3	P4	P5
MS Motif #2:	F	H	F	F	K
	Y	N		Y	R
	W	Q		W	

-continued

Position	P1	P2	P3	P4	P5
V	F		V		
L			L		
I			I		
A			A		
M			M		

The third sequence motif represented a modification of the TCR contact residues preferred by a subgroup of MBP (85-99) specific clones. For these clones, P5 (Lys 93) was absolutely conserved while P3 (Phe 91) could be substituted by some aromatic or aliphatic amino acids. The third motif for multiple sclerosis antigens was defined as:

Position	P1	P2	P3	P4	P5
MS Motif #3:	V	H	F	F	K
	L	F	Y	Y	
	I	Y	W	W	
	A	W	V	V	
	M		L	L	
			I	I	

These HLA-DR motifs also matched well with the structural requirements for an HLA-DQ1 restricted clone specific for the MBP(85-99) peptide. This clone required the same minimal peptide segment as DR2 restricted clones (residues 87-97). As in the DR2 restricted clones, P2 (His 90), P3 (Phe 91) and P5 (Lys 93) appeared to be the primary TCR contact residues. Substitution of these hydrophobic positions by aspartic acid greatly diminished the stimulatory capacity of the peptide while substitutions by other hydrophobic amino acids were tolerated. These data suggest that the MBP (85-99) peptide is bound in a similar fashion to HLA-DR2b and to HLA-DQ1 and that the same peptide residues are critical for interaction with the TCR.

These motifs were used as search criteria in a search of protein databases (PIR and SwissProt) using the Genetics Computer Group software (program: findpatterns). More than 600 sequences of viral and bacterial origin were identified that matched these criteria. From this set, sequences were selected based on the following criteria: (1) Viruses known to cause human pathology, (2) Viruses prevalent in the Northern Hemisphere where MS occurs most frequently, (3) Selected bacterial sequences associated with inflammatory CNS disease (such as *Borrelia burgdorferi*) and with invasive infections (such as *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Not included were most viruses that cause infections in tropical countries, sequences derived from vaccinia virus and a large number of sequences from *E. coli* (which is part of the normal intestinal flora). When multiple antigenic variants were present, one or several sequences that best fit the motifs were chosen. The selected peptides were synthesized by Pin-Technology on a 1 mg scale (Chiron Mimotopes, San Diego). Seventy peptides were made which fit motifs #1 and #2 and 59 peptides for motif #3.

These peptides were then tested for their ability to activate human MBP(85-99) specific T cell clones that had been previously established from blood T cells of two patients with relapsing-remitting MS (Wucherpfennig et al., 1994a; Wucherpfennig et al., 1994b). Homozygous B cell lines that expressed DR2 (DRB1*1501 or DRB1*1602) or DQ1 were used as antigen presenting cells (APCs) in these T cell proliferation experiments (Wucherpfennig et al., 1994a). As a positive control, all clones were shown to be activated by

the MBP(85-99) peptide. Seven clones were tested with the viral/bacterial peptides selected according to the sequence motifs.

Three of the seven clones tested were efficiently activated by several viral/bacterial peptides. The first clone (Hy.1B11) was HLA-DQ1 restricted whereas the other two clones (Hy.2E11 and Hy.1G11) were HLA-DR2 restricted (Wucherpfennig et al., 1994a). Among the 70 peptides selected according to motifs #1 and #2, three mimicry peptides stimulated the DQ1 restricted clone and two peptides stimulated both of the DR2 restricted T cell clones. Among the group of 59 peptides selected according to motif #3, one peptide was identified for the DQ1 restricted clone while two were identified for the DR2 restricted clones.

Taken together, the DQ1 restricted T cell clone recognized five structurally related peptides: the immunodominant MBP(85-99) peptide, three viral peptides (from Herpes simplex, Adenovirus type 12 and Human Papillomavirus) and a bacterial peptide (*Pseudomonas aeruginosa*). Two of the DR2 restricted clones were activated by four peptides. Both clones recognized the MBP(85-99) peptide as well as peptides from EBV and Influenza virus. In addition, one clone recognized a viral peptide from Reovirus (clone Hy.2E11) and one recognized a peptide from Herpes simplex virus (clone Hy.1G11). These results, and the sequences of these peptides, are summarized below in Table 2.

TABLE 2

Peptides Recognized by Clone Hy.1B11 (DQ1 Restricted):

MBP(85-99)	ENPVVHFFKNIVTPR
Herpes Simplex, UL15 Protein	FRQLVHFVRDFAQLL
Adenovirus Type 12, ORF	DFEVVTFILKDVLPF
<i>Pseudomonas aeruginosa</i> , Phosphomannomutase	DRLLMLFAKDVVSRN
Human Papillomavirus Type 7, L2 Protein	IGGRVHFFKDISHIA

Peptides Recognized by Clone Hy.2E11 (DR2 Restricted):

MBP(85-99)	ENPVVHFFKNIVTPR
Epstein-Barr Virus, DNA Polymerase	TGGVYHFFVKKHVHES
Influenza Type A, Hemagglutinin	YRNLVWFIIKKNTRYP
Reovirus Type 3, Sigma 2 Protein	MARAALFKTVVGFGG
Peptides Recognized by Clone Hy.1G11 (DR2 Restricted):	
MBP(85-99)	ENPVVHFFKNIVTPR
Epstein-Barr Virus, DNA polymerase	TGGVYHFFVKKHVHES
Influenza Type A, Hemagglutinin	YRNLVWFIIKKNTRYP
Herpes Simplex, DNA Polymerase	GGRRLFVFKAHVRES

For these viral/bacterial peptides to be involved in the initiation of autoimmunity, they have to be capable of potent T cell stimulation that results in marked clonal expansion of autoaggressive T cell clones. The stimulatory capacity of each of these peptides was therefore compared to the MBP (85-99) peptide in a titration experiment. The peptides were found to be efficient stimulators of the MBP specific T cell clones; in particular the EBV peptide (DR2 restricted clones) and the adenovirus peptide (DQ1 restricted clone) were similar to the MBP(85-99) peptide in their stimulatory capacity. These results demonstrate that the T cell activation is not the result of a minor degree of 'cross-reactivity' but rather the result of structural similarity sufficient for potent T cell activation.

Comparison of the peptide sequences that were found to stimulate the same TCR revealed several interesting points:

(1) Only one peptide (Human Papillomavirus L2 protein) had striking sequence similarity with the MBP(85-99) peptide in that all amino acids in the MBP(85-95) segment except position 94 (Asn to Asp) were identical (Table 2). For all other sequences, simple alignment

would not have predicted them to be efficient stimulators of MBP(85-99) specific T cell clones. Therefore, absent the method of the present invention, these peptides would not have been identified.

- (2) At positions not specified by the search criteria, the selection for particular amino acids was still apparent (Table 2). For the DQ1 restricted clone, for example, aspartic acid was selected at position P6 (residue 94 of MBP), a probable TCR contact residue, in all four peptides. This position is occupied by asparagine in the MBP peptide (similar size, but no negative charge). Substitution of Asn 94 for Asp in the MBP peptide markedly increased its stimulatory capacity for the DQ1 restricted clone but reduced it for the DR2 restricted clone. Selection also occurred at the neighboring P7 MHC contact (Ile 95) for which Ile, Val or Phe were selected (all hydrophobic).
- (3) Different selection events occurred for the DQ1 and the DR2 restricted clones: At position P6 (94 of MBP), there was selection of aspartic acid (negative charge) for DQ1 peptides whereas there was selection for lysine (positive charge) in two of the three peptides presented by DR2.
- (4) In the flanking segments (residues 85-87 and 97-99), no apparent selection took place as amino acids with different size and charge were allowed.

The majority of the viruses from which these peptides were identified are common human pathogens: Influenza Type A frequently causes respiratory tract infections; Human Papillomavirus infects epithelial tissues and has been linked to cervical carcinomas; and Epstein-Barr Virus (EBV) causes an acute viral syndrome (infectious mononucleosis) in young adults. Human Herpesvirus 1 (Herpes simplex), EBV and Human Papillomavirus cause latent or persistent infections with neurons (Herpes simplex), B cells (EBV) and epithelial cells (Papillomavirus) serving as the reservoirs. Viral expression can be reactivated by UV exposure and stress (Herpes simplex) and by B cell activation (EBV) (Schwarz et al., 1985; Epstein et al., 1977; Spruance 1985; Tovey et al., 1978). For the induction and maintenance of an autoimmune response these persistent viral infections are of particular interest as they could explain the chronicity of the clinical disease and the clonal expansion and persistence of MBP specific T cells. Reactivation of viral expression may also be involved in triggering clinical relapses. By this mechanism, viral peptides could activate resting MBP specific T cells in periphery and allow them to invade the CNS.

Are these foreign epitopes actually presented to autoreactive T cells during a viral infection? The peptide from the EBV DNA polymerase allowed this question to be addressed. In EBV transformed B cells (which were used as antigen presenting cells in the T cell assays) the lytic viral cycle is repressed. The DNA polymerase gene is not transcribed in this latent state; however, B cell activation results in activation of the lytic cycle and in the expression of the DNA polymerase gene (Datta et al., 1980). To examine MHC class II restricted presentation of the EBV DNA polymerase, an HLA-DR2⁺ EBV transformed B cell line (MGAR) and an MHC mismatched control (9001, HLA-DR1) were pretreated for 36 hours with phorbol ester which was removed by extensive washing prior to coculture of APC with T cells. T cell clones Hy.2E11 and Hy.1G11, which recognize the EBV DNA polymerase peptide presented by HLA-DR2, were activated by an HLA-DR2⁺ EBV transformed B cell line pretreated with phorbol ester. This effect was specific because MHC mismatched B cells did not activate the clones; also, a control clone (Ob.1A12) that

recognized MBP(85-99) but not the EBV peptide was not activated. In a separate experiment, T cell activation was blocked by a mAb specific for HLA-DR (mAb L243) but not by a mAb specific for HLA-DQ (G2a.5). These results demonstrate that the MBP specific T cell clones recognize not only the viral peptide but also antigen presenting cells infected with the virus. In vivo this recognition event could lead to chronic antigenic stimulation of MBP specific T cells as B cell activation results in the expression of EBV genes, including the DNA polymerase gene.

Finally, the presentation of the viral peptides by different DR2 subtypes was compared to determine if they are efficiently presented by the disease associated molecule (DRB1*1501, the most common DR2 subtype). The MBP peptide was presented by three of the four DR2 subtypes; the peptide was not presented by DRB1*1601 which differs from DRB1*1602 by a single amino acid substitution (at position DRB67, a possible TCR contact). The two viral peptides were presented much better by the DR15 molecules (DRB1*1501 and 1502 which differ only at position DRB86) than by DRB1*1602. This was particularly evident for the influenza peptide which only activated the T cell clone when presented by the DRB1*1501/1502 molecules but not by DRB1*1602. These results indicate that the viral peptides identified herein are preferentially presented by the MS associated DR2 molecule (DRB1*1501).

DEFINITIONS

For clarity of interpretation and to clearly and distinctly point out the subject matter of the claimed invention, the following definitions are provided for several terms used in the claims appended hereto.

The term "sequence motif," in accordance with the description provided herein, means a series of restrictions on the residues which may occupy certain relative positions of an amino acid sequence. A sequence motif must restrict at least three and preferably four or five positions of an amino acid sequence. The relative positions of the first (N-terminal) and last (C-terminal) restricted amino acid positions shall be separated by at least two but no more than twelve amino acid residues. For example, P1 and P4 may be the first and last restricted residues and these residues are separated by two residues. As another example, P-1 and P11 may be the first and last restricted residues and these are separated by ten residues. Positions between the first and last restricted positions may be restricted or unrestricted with the exception that a total of at least three positions of the motif must be restricted. Of the three positions which must be restricted, at least two must be residues corresponding to major MHC binding pockets. If only two of the restricted residues correspond to MHC binding residues, the third must correspond to a TCR contact residue. Further, at least one of the positions restricted must correspond to either the P1 or P4 binding position. By "restricted" is meant that at least one, and preferably ten, amino acid residues shall be excluded from a position.

An amino acid sequence "corresponds" to a sequence motif if it can be aligned with the positions of the sequence motif such that, at each restricted position of the motif, the amino acid sequence includes a residue which is not excluded from that position by the restrictions which define the motif. As the restrictions which define the motif are derived from the size and nature of the MHC binding pockets of HLA-DR proteins and, optionally, the TCR contact residues of known epitopes, the restricted positions of the binding motif may also be said to correspond to the MHC binding pockets and TCR contact residues.

The term "isolated," in reference to a protein or polypeptide, means separated from its native or natural chemical microenvironment. Thus, a polypeptide isolated from a bacterium shall be in a preparation substantially free of most of the other bacterial polypeptides and, similarly, an isolated viral polypeptide preparation shall be substantially free of the other polypeptides which comprise the virus.

The term "associated with," as used in connection with a particular HLA-DR protein and an autoimmune disease or autoimmune response, shall mean that the protein and the disease/response have been positively correlated by clinical or epidemiological studies such that the likelihood of developing the disease/response has been shown to be increased by presence of the protein.

The term "HLA-DR protein" means the particular protein product of a particular allele of the MHC class II HLA-DR gene. A disease associated with an HLA-DR protein is one associated with such a particular protein and not merely with the HLA-DR gene locus.

By the term "human pathogen" is meant a bacterium, virus or protozoan capable of infecting humans and generating an immune response. The term specifically is intended to exclude the bacteria which form part of the normal human intestinal flora. By this term, "normal human intestinal flora," is meant the bacteria which normally inhabit the human gut, such as *Escherichia coli*, but which do not normally cause disease.

By the term "autoreactive," as applied to T cells, is meant T cells from a human which are activated by a human self epitope. By "activation" of T cells is meant induction to proliferate, secrete lymphokines (cytokines) and/or to initiate effector activity (e.g. cytotoxicity).

By the term "autoantigen" is meant a self protein or polypeptide which includes a "self epitope." By "self epitope" is meant that part of an autoantigen which is recognized by T cells when bound to and presented by an MHC molecule.

By the term "effective amount," with respect to tolerizing an individual to an antigen, is meant an amount of the antigen sufficient to render T cells, otherwise specific for the antigen, unresponsive to the antigen when bound to and presented by an MHC molecule. T cells which are unresponsive fail to activate when presented with the antigen for which they are specific. By the term "effective amount," with respect to immunizing an individual to an antigen, is meant an amount sufficient to induce an immune response which results in T cells specific for the antigen. Typical ranges of dosages are from 1 nanogram/kilogram to 100 milligrams/kilogram or even 500 milligrams/kilogram. Effective amounts will vary according to such factors as age, sex and sensitivity to the antigen.

By the term "core MHC binding residues" is meant the residues of an epitope corresponding to the P-1 to P₉ positions of a peptide bound to an HLA-DR molecule. By the term "inner core MHC binding residues" is meant those residues of an epitope corresponding to the P-1 to P₆ positions of a peptide bound to an HLA-DR molecule.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1 1 1) NUMBER OF SEQUENCES: 16

(2) INFORMATION FOR SEQ ID NO:1:

5,874,531

31

32

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Tbr Pro Met Phe Leu Leu Ser Arg Asn Thr Gly Glu Val Arg Tbr
1 S 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL NO

(v) FRAGMENT TYPE: internal

(v) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(x) SEQUENCE DESCRIPTION: SEQ ID NO:S:

Cys Glu Cys Asn Ile Lys Val Lys Asp Val Asn Asp Asn Phe Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(i) MOLECULE TYPE: peptide

(111) HYPOTHETICAL NO

(v) FRAGMENT TYPE: internal

(v) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(*) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Ala Arg Thr Leu Asn Asn Arg Tyr Thr Gly Pro Tyr Thr Phe
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(1) MOLECULE TYPE: peptide

(1) HYPOTHETICAL NO

(v) FRAGMENT TYPE: internal

(v 1) ORIGINAL SOURCE:

Gln Ser Gly Thr Met Arg Thr Arg His Ser Thr Gly Gly Thr Asn

4.3.3 INFORMATION FOR SEQ ID NO:8:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) TOPOLOGY: linear

-continued

(1) MOLECULE TYPE: peptide

(1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: HERPES SIMPLEX

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe	Arg	Gln	Leu	Val	His	Phe	Val	Arg	Asp	Phe	Ala	Gln	Leu	Leu
1														15
						5								

(2) INFORMATION FOR SEQ ID NO:9:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: ADENOVIRUS TYPE 12

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp	Phe	Glu	Val	Val	Tbr	Phe	Leu	Lys	Asp	Val	Leu	Pro	Glu	Phe
1														15
						5								

(2) INFORMATION FOR SEQ ID NO:10:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: PSEUDOMONAS AERUGINOSA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp	Arg	Leu	Leu	Met	Leu	Phe	Ala	Lys	Asp	Val	Val	Ser	Arg	Asn
1														15
						5								

(2) INFORMATION FOR SEQ ID NO:11:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: HUMAN PAPILLOMAVIRUS TYPE 7

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile	Gly	Gly	Arg	Val	His	Phe	Phe	Lys	Asp	Ile	Ser	Pro	Ile	Ala
1														15
						5								

-continued

(2) INFORMATION FOR SEQ ID NO:12:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v 1) ORIGINAL SOURCE:

(A) ORGANISM: EPSTEIN-BARR VIRUS

(x 1) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Thr	Gly	Gly	Val	Tyr	His	Phe	Val	Lys	Lys	His	Val	His	Glu	Ser
1				5									10	15

(2) INFORMATION FOR SEQ ID NO:13:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v 1) ORIGINAL SOURCE:

(A) ORGANISM: INFLUENZA VIRUS TYPE A

(x 1) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Tyr	Arg	Asn	Leu	Val	Trp	Phe	Ile	Lys	Lys	Asn	Thr	Arg	Tyr	Pro
			5										10	15

(2) INFORMATION FOR SEQ ID NO:14:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v 1) ORIGINAL SOURCE:

(A) ORGANISM: REOVIRUS TYPE 3

(x 1) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met	Ala	Arg	Ala	Ala	Phe	Leu	Phe	Lys	Thr	Val	Gly	Phe	Gly	Gly
1					5				10				15	

(2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: peptide

(1 1 1) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

-continued

(v) ORIGINAL SOURCE:
 (A) ORGANISM: HERPES SIMPLEX VIRUS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly	Gly	Arg	Arg	Leu	Phe	Phe	Val	Lys	Ala	His	Val	Arg	Glu	Ser
1				5				10				15		

(2) INFORMATION FOR SEQ ID NO:16:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(1 i) MOLECULE TYPE: peptide

(1 ii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu	Asn	Pro	Val	Val	His	Phe	Phe	Lys	Asn	Ile	Val	Thr	Pro	Arg
1			5					10				15		

We claim:

1. An isolated polypeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, and SEQ ID NO.: 7.
2. An isolated polypeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID

NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, and SEQ ID NO.: 15.

3. A composition comprising a pharmaceutically acceptable carrier and a polypeptide of claim 1.

4. A composition comprising a pharmaceutically acceptable carrier and a polypeptide of claim 2.

35

* * * * *

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A Division of Harcourt Brace & Company

The Curtis Center
Independence Square West
Philadelphia, Pennsylvania 19106

Library of Congress Cataloging-in-Publication Data

Abbas, Abul K.

Cellular and molecular immunology / Abul K. Abbas, Jordan S. Pober,
Andrew H. Lichtman. — 2nd ed.

p. cm.

Includes bibliographical references and index.

ISBN 0-7216-5505-X

1. Cellular immunity. 2. Molecular immunology. I. Lichtman,
Andrew H. II. Pober, Jordan S. III. Title. IV. Title: Cellular
and molecular immunology.

[DNLM: 1. Immunity, Cellular. 2. Lymphocytes—immunology. QW

568 A122c 1994]

QR185.5.A23 1994

616.07'9—dc20

DNLM/DLC

93-50216

Cellular and Molecular Immunology, 2nd edition

ISBN 0-7216-5505-X

I.E. ISBN 0-7216-5290-5

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Printed in the United States of America.

Last digit is the print number: 9 8 7 6 5 4 3

T lymphocytes play a central role in specific immune responses to protein antigens. In Chapter 5, we introduced the concept that the physical forms of antigens recognized by T cells are actually peptide fragments that are derived from protein antigens and are bound to cell surface proteins encoded by genes of the major histocompatibility complex (MHC). Peptides bound to class I MHC molecules are typically derived from proteins synthesized in the cell that displays them (i.e., "endogenous antigens") and are recognized by CD8⁺ T cells, which are usually cytolytic T lymphocytes (CTLs). CTLs provide a major host defense mechanism against intracellular microbes. In contrast, peptides derived from proteins in the extracellular environment ("exogenous antigens") are displayed in association with class II MHC molecules and are recognized by CD4⁺ T cells, which are usually helper T lymphocytes. Helper T cells are required for the induction of humoral and cell-mediated responses, which are most effective in eliminating extracellular pathogens. In this chapter, we will describe in greater detail the characteristics of the peptide-MHC molecule complexes, the nature of the cells that form and display these complexes, and the mechanisms by which cells convert endogenous and exogenous protein antigens to peptides that can bind to MHC molecules. The activation and effector mechanisms of T cell subsets that occur subsequent to recognition of peptide-MHC complexes will be discussed in later chapters.

CHARACTERISTICS OF ANTIGEN RECOGNITION BY T LYMPHOCYTES

Our current understanding of T cell antigen recognition is the culmination of a vast amount of work that began with studies of the physicochemical forms of antigens that stimulated cell-mediated immunity. These studies led to the discovery that cells other than T lymphocytes play an obligatory role in T cell activa-

tion by foreign antigens, and later to the elucidation of the function of MHC molecules in T cell antigen recognition.

The Forms of Antigens Recognized by T Lymphocytes

The specificity of T lymphocytes for complexes of peptides and MHC molecules determines several characteristics of T cell antigen recognition, which differ in fundamental ways from antigen recognition by antibody molecules.

1. *T lymphocytes recognize only peptides*, whereas B cells can specifically recognize peptides, proteins, nucleic acids, polysaccharides, lipids, and small chemicals. As a result, T cell-mediated immune responses are induced only by protein antigens (the natural source of foreign peptides), whereas humoral immune responses are seen with protein and non-protein antigens. Some T cells are specific for chemically reactive forms of haptens such as dinitrophenol. In these situations, it is likely that the haptens bind to cell surface proteins, including MHC molecules, and peptides containing these hapten conjugates are recognized by T cells.

2. *T cells recognize only linear determinants of peptides defined predominantly by primary amino acid sequences* that assume extended conformations within the peptide-binding clefts of MHC molecules. In contrast, B cells specific for protein antigens may recognize conformational determinants that exist when proteins are in their native tertiary (folded) configuration or determinants that are exposed by denaturation or proteolysis. Thus, when an animal is immunized with a native protein, the antigen-specific T cells that are stimulated will respond to denatured or even proteolytically digested forms of that protein. In contrast, antibodies produced by B cells after immunization with the native protein react only with the native protein (Table

TABLE 6-1. Qualitative Differences in Antigen Recognition by T and B Lymphocytes

Immunizing Antigen	Secondary Antigen Exposure	Secondary Immune Response	
		B Cell-Mediated (Antibody Production)	T Cell-Mediated (Delayed-Type) Hypersensitivity
Native protein	Native protein	+	+
Denatured protein	Native protein	-	+
Native protein	Denatured protein	-	+
Denatured protein	Denatured protein	+	+

Antigen recognition by T and B lymphocytes is qualitatively different. In an immunized animal, B cells are specific for conformational determinants of the immunogen and, therefore, distinguish between native and denatured protein antigens. T cells, however, do not distinguish between native and denatured protein antigens because T cells recognize linear epitopes on short peptides derived from the intact proteins by proteolysis.

6-1). Consistent with this difference in the nature of antigenic determinants for T and B cell recognition is the finding that T cell responses to a soluble antigen cannot be inhibited using antibodies specific for conformational determinants of that antigen, whereas antigen recognition by B cells can be competitively inhibited by such antibodies.

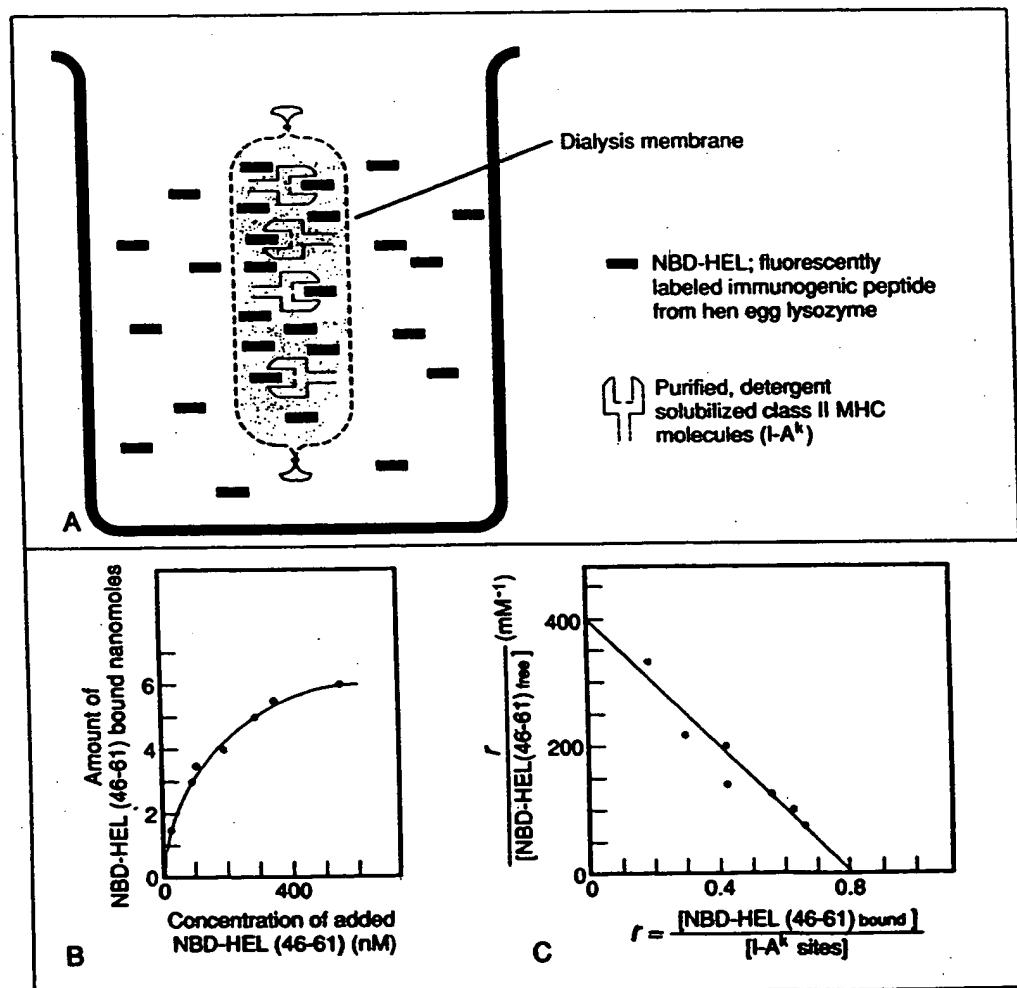
3. *T cells recognize and respond to foreign peptide antigens only when the antigen is attached to the surfaces of other cells*, whereas B cells and secreted antibodies bind soluble antigens in body fluids or cell surface antigens. This is because MHC molecules form part of the complex that T cells recognize, and these molecules are cell surface-bound integral membrane proteins. The display of peptide-MHC complexes in a form that can be recognized by T cells is called **antigen presentation**. Cells that display antigens in this form are called **antigen-presenting cells (APCs)**. The properties and functions of APCs are discussed later in the chapter. Although historically the term APC has most often been used to describe cells that present antigen to CD4⁺ helper T lymphocytes, it is now appropriate to describe target cells of CTL lysis as APCs as well, since CTLs also recognize peptide-MHC complexes on the surface of these target cells.

Physicochemical Features of Peptide-MHC Complexes

In order to stimulate T cell responses, peptides derived from protein antigens must bind to MHC molecules and must remain stably bound long enough to allow specific T cells to engage the complex. We will first describe the characteristics of the peptide-MHC complexes that T cells recognize, and later we will describe how these complexes are formed. The structural basis for peptide binding to both class I and class II MHC molecules has been analyzed by three approaches: (1) binding assays of peptides to purified MHC molecules in cell-free solutions; (2) x-ray crystallographic analyses of purified MHC molecules with bound peptides; and (3) amino acid analysis of peptides eluted from MHC molecules purified from cell membranes. The following are the main features of MHC-peptide interactions elucidated by these approaches:

1. *The association of antigenic peptides and MHC molecules is a saturable, low-affinity interaction ($K_d = 10^{-6} M$) with a slow "on rate" and a very slow "off rate."* These features were determined first by the techniques of equilibrium dialysis (see Chapter 3 and Fig. 6-1) and

FIGURE 6-1. Demonstration of peptide binding to MHC molecules by equilibrium dialysis. Purified I-A^k class II MHC molecules bind a peptide fragment of hen egg lysozyme (HEL), HEL(46–61), that is labeled with a fluorescent marker (NBD), which allows the concentration of the peptide to be determined (A). Analysis of the amount of HEL bound (i.e., the concentration within the dialysis membrane minus the concentration outside) at varying concentrations of peptide shows that the binding is saturable (B). Scatchard analysis shows that each I-A^k molecule has approximately one binding site (X-axis intercept), and the dissociation constant (K_d) is approximately $1 \times 10^{-6} M$ (calculated from the slope of the line). In this plot, r represents the number of peptide molecules bound to each MHC molecule when there is an excess of peptide (C). Note that HEL(46–61) is known to be presented in association with I-A^k molecules. (Modified with permission from Babbitt, B. P., P. M. Allen, G. Matsueda, E. Haber, and E. R. Unanue. Binding of immunogenic peptides to *Ia* histocompatibility molecules. *Nature* 317:359–361, 1985. Copyright © 1985, Macmillan Magazines Ltd.)



10-29-02

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**TRANSMITTAL
FORM**

Application Serial Number	08/991,628
Filing Date	November 5, 1997
First Named Inventor	Strominger
Group Art Unit	1644
Examiner Name	M. DiBrino
Attorney Docket No.	HAR-001DV
Patent No.	Not applicable
Issue Date	Not applicable

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ENCLOSURES (check all that apply)

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| <input type="checkbox"/> Amendment/Response <ul style="list-style-type: none"> <input type="checkbox"/> Preliminary <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Letter to Official Draftsperson including Drawings [Total Sheets ____] | <input type="checkbox"/> Request For Continued Examination (RCE) Transmittal <ul style="list-style-type: none"> <input type="checkbox"/> Power of Attorney (Revocation of Prior Powers) | <input type="checkbox"/> Status Inquiry <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Return Receipt Postcard <input type="checkbox"/> Certificate of First Class Mailing under 37 C.F.R. 1.8 |
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APPLICANT(S): Strominger et al.

SERIAL NO.: 08/991,628

GROUP NO.: 1644

FILING DATE: November 5, 1997

EXAMINER: DiBrino, M.

TITLE: Preparations for Inducing Immunotolerance and Uses Therefor

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Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO COMMUNICATION

Sir:

This paper is responsive to the communication mailed from the U.S. Patent and Trademark Office on October 2, 2002. The communication required that Applicants submit additional copies of the appeal brief previously submitted on February 11, 2002. Applicants enclose three copies of that appeal brief.

Applicants believe this submission completes all formalities of 37 C.F.R. § 1.192.

Applicants do not believe that any petition or fee is required at this time. Nevertheless, please charge any required fee to Deposit Account No. 20-0531, and if any petition is required, please consider this paper to be a conditional petition therefor.

Respectfully submitted,

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